FERTILITY OF FROST BOILS AND THE EFFECT OF DIAPIRISM ON PLANT NITROGEN UPTAKE IN A POLAR DESERT ECOSYSTEM OF THE CANADIAN HIGH ARCTIC

A Thesis Submitted to the College of
Graduate Studies and Research
In Partial Fulfillment of the Requirements
For the Degree of Master of Science
In the Department of Soil Science
University of Saskatchewan
Saskatoon

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ABSTRACT

Polar desert environments are limiting in plant available nutrients, mainly nitrogen (N) and phosphorus (P) that severely limit plant growth and establishment. Cryogenic activity regularly patterns the ground into a patchwork of frost boils – sorted circles that are associated with an increase in moisture, fertility and plant cover. Within some frost boils, the accumulation of ice-rich soil at the permafrost table can cause an upward flow of soil organic carbon (SOC) enriched permafrost material into the active layer. These diapiric intrusions are predicted to fuel microbial activity and enrich the horizon in N and P; however, the enrichment of the diapir horizon and accessibility by plants has yet to be studied. The aim of this research was to characterize the N distribution within diapir horizons located in frost boils and the effect of these intrusions on vascular plant N uptake in a polar desert ecosystem of the Canadian High Arctic. Natural abundance and enriched isotope ^{15}N techniques were used to trace the flow of N through the soil-plant system. Surface and diapir horizons contained the highest total C and total N content within frost boils. Natural abundance $\delta^{15}N$ analysis indicated that uptake by *Salix arctica* plants located on frost boils in the absence of a diapir horizon were sourcing N from the surface. However, when diapir nutrients became available, *S. arctica* plants began sourcing N from the diapir horizon and underlying low SOC sources in the soil, while reducing uptake from the surface. The altered foraging strategy of *S. arctica* in response to diapir horizon formation was further indicated by significant uptake of atom%^{15}N nutrients that were injected directly into diapir horizons. These findings suggest diapir horizons are enriched in N and accessible by plants roots as an important nutrient source that is instrumental in their survival within frost boils of a polar desert ecosystem in the high arctic.

ACKNOWLEDGEMENTS

I am immensely grateful for the guidance and support of my supervisor, Dr. Steven Siciliano, as well as for providing numerous opportunities for exceptional and unforgettable seasons of field work. I would like to thank my advisory committee Dr. Ken Van Rees and Dr. Eric Lamb for their advice, support and input during all aspects of this project. I also thank Dr. Darcy Henderson for his contributions as an external examiner.

Financial support was provided by the Natural Sciences and Engineering Research Council (NSERC) and the Northern Scientific Training Program (NSTP). The Polar Continental Shelf Project at Resolute Bay provided logistical support during fieldwork.

This thesis would never have been possible without the extraordinary fieldwork of the Arctic Field Crew – Amanda Guy, Martin Brummell, Mitsuaki Ota, and Nicole Marleau - as well as Dr. Siciliano and Dr. Van Rees for assistance and inspiration in the field. Further endless thanks to Amanda Guy for collecting and providing the surface cover and vis-NIR field data used within this thesis. Marvin - I thank you for not eating me. Many thank you's to everyone in the Soil Environmental Toxicology Lab, especially the patient presence and knowing work of Richard Nhan. Thank you to the many helping hands within the Soil Science Department and Myles Stocki for performing the mass spectrometry analysis.

I am very grateful to my parents, Calvin and Cindy, and my siblings, Danielle and Sam, for their unfailing support. Finally - to Kyle - thank you for sharing your endless encouragement, warmth and positivity. You are always the root of my inspiration.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

APM	Ammonium phosphomolybdate
DON	Dissolved organic nitrogen
IRMS	Isotope ratio mass spectrometer
$^{18}O_P$	$^{18}O/^{16}O$ isotope ratio of O bound to P_i
MAP	Magnesium ammonium phosphate
P_i	Inorganic phosphorus
SOC	Soil organic carbon
SOM	Soil organic matter
Vis-NIR	Visible and near infrared spectrometer
VSMOW	Vienna standard mean ocean water

1. INTRODUCTION

The arctic region is changing as temperature and precipitation regimes are altered and perennially frozen soils are becoming active (IPCC, 2013). These changes are exacerbated at the northernmost latitudes – an area described as the High Arctic – that occurs primarily as a barren landscape of weakly-developed soils and low to non-existent plant cover (Bliss and Matveyeva, 1992). Polar desert landscapes are covered by patterned ground features, often as a patchwork of circles – termed frost boils – that are formed by cryogenic activity as the soil freezes and thaws (i.e. cryoturbation) (Ping et al., 2015). Frost boil features are associated with increasing fine particle development, moisture, and biological activity that increases the accumulation of plant-available nutrients in the soil (Walker et al., 2004; Ugolini et al., 2006; Frost et al., 2013). As these landscapes are extremely nutrient-limited, particularly in N and P (Madan et al., 2007), frost boils offer a haven for establishment and growth in the otherwise harsh soil environment (Sutton et al., 2006; Makoto and Klaminder, 2012). Although the association of increasing fertility and plant cover within these features is well-established (Walker et al., 2008), the distribution of N and P in the belowground soil environment and the processes controlling their availability for uptake that is necessary for plant survival remain largely unknown.

Nutrient inputs are low in polar deserts and occur at the surface from N_2-fixation by organisms in biological soil crusts (Stewart et al., 2011a) and vascular plant litter decay (Bliss et al., 1984). Belowground stocks of SOC are relatively large and much is stored away within the

perennially frozen permafrost layer (Michaelson et al., 2008; Palmtag et al. 2015). Cryoturbation processes can redistribute this material into the active layer as warming soil temperatures thaw the permafrost and meltwater accumulates at the permafrost table to create an area of ice-rich soil. When the ice-rich soil becomes lighter than the overburden, the soil can sink and cause a diapiric injection of SOC into the active layer (Swanson et al., 1999). As temperatures increase, the SOC is predicted to fuel microbial activity and mineralization of nutrients (Wild et al., 2014). However, the ability of the redistributed SOC to act as a source of plant-available N and P has yet to be studied.

Polar desert soils are projected to become more cryogenically active as air temperatures warm and increasing meltwater flow from a larger snow pack cause deeper thaw depths and substantial degradation of the permafrost (Callaghan et al., 2005; IPCC, 2013). In this environment, diapirism is likely to become more prevalent within frost boils across the landscape. The objective of this thesis was to determine whether diapirism increased plant-available N and P in frost boils within the polar desert ecosystem. This is the first examination of the potential of diapir horizons to act as an instrumental nutrient source in polar desert plant survival with the aim of predicting what this relationship might offer in a changing climate. If plants are able to access these enriched SOC sources, the surface of polar deserts have the potential to change considerably as enhanced N and P fertility may facilitate greater growth and establishment in these unproductive ecosystems (Madan et al., 2007; Arens et al., 2008).

This thesis consists of five chapters, beginning with the introduction (Chapter 1) and followed by the literature review (Chapter 2). Prepared in manuscript format, Chapter 3 is a lone research study that determined the soil-plant N dynamics in a frost boil ecosystem located at a polar desert field site. Following the research study is a summary of the findings and future

research directions regarding N dynamics (Chapter 4). The original objective of the study was to also characterize the soil-plant P dynamics within the diapir horizon, however methodological difficulties with isotopic purification techniques prevented the completion of the laboratory P analysis. A detailed description of the laboratory methods used, complications that arose, and recommendations for improving P isotopic analysis are outlined in Appendix A1.

2. LITERATURE REVIEW

2.1 Polar Desert Ecology

Polar desert and semidesert regions cover approximately 40% of arctic land - an area of 2.21 x 10^6 km^2– the majority of which occurs in North American regions of the Canadian Arctic Archipelago and Greenland (Bliss and Matveyeva, 1992). They are barren expanses of young, poorly developed soil (<12 000 yr) with an active layer, as thin as 0.2 m (Bliss, 1997), overlaying a continuous, impermeable permafrost layer that is usually within 1 m of the soil surface (Bockheim, 2015). Soils are classified as Regosolic Static or Turbic Cryosols (Soil Classification Working Group, 1998) depending on the degree of cryoturbation (any soil movement from frost action) that occurs during annual freeze and thaw cycles. Cryoturbation causes sorting of soil based on particle size into patterned ground formations visible on the soil surface (Figure 2.1), as well as mixing and distortion of horizons and redistribution of soil organic carbon (SOC) deeper into the profile (Bockheim and Tarnocai, 1998; Ping et al., 2008). Arctic regions are estimated to hold half of the globe's SOC with up to 88% frozen in permafrost (Tarnocai et al., 2009); however, regional estimates are lacking (Campeau et al., 2014) and the amount in permafrost of polar desert soils is unclear but likely much less than other areas of the arctic tundra (Michaelson et al., 2008; Palmtag et al. 2015). Mean annual temperature ranges from -7 to -19°C and precipitation is low, <100 mm to 300 mm that falls primarily as snowfall (Bliss, 1997). Over the last century this region has changed rapidly and substantial degradation

Figure 2.1. A polar semi-desert site at Alexandra Fiord, NU, CA (78°51'N, 76°06'W). Soils are sorted into patterned ground features from cryoturbation processes that affect the distribution of plant cover on the landscape.

of near-surface permafrost and increases in winter snowfall and annual temperatures of 2 to 8°C are predicted by the end of the 21st century (IPCC, 2013).

Polar deserts are defined by their low plant productivity with vascular plant cover less than 5%, increasing up to 20% in semideserts (Figure 2.1) (Bliss and Matveyeva, 1992). The short growing season – lasting 1.5 to 2.5 months – is cold with mean July temperatures between 3 to 8 °C, dry, and often windy, limiting the growth and development of plants (Bliss, 1997; Bliss and Gold, 1999). Life cycles are long and many depend upon stored resources to grow and long flowering cycles, vegetative propagation or clonal growth to reproduce (Callaghan et al., 2005). However, recruitment from seed is more common here than in other arctic habitats (Hobbie, 2007). Many species adapt to the extreme environment with slow-growing, low, compact forms - such as cushion plants that are able to minimise wind and ice damage and create

a thermal pocket that can exceed air temperature by up to 20°C (Mølgaard, 1982; Gold, 1998). In the Canadian High Arctic, less than 20 vascular plant species are able to establish in deserts with the most common being *Draba Corymbosa, D. subcapitata, Papaver radicatum, Saxifraga oppositifolia, Puccinellia angustata* and *Minuartia rubella* (Bliss and Matveyeva, 1992; Walker et al., 2005). The development of a thin (2 to 3 mm) biological soil crust of bryophytes, lichens and N- fixing cyanobacteria species (mainly *Nostoc commune*) increases in the moist surfaces of snowflush or water seepage areas, along with the deciduous shrub *Salix arctica* and sedges such as *Luzula confusa*. In semideserts, or regions with low but greater than 5% plant cover, plant communities of dwarf-shrub cryptogram dominated by *Dryas integrifolia* and associations with *S. arctica, S. oppositifolia, D. corymbosa, Cerastium alpinum* and *P. radicatum* develop on well-drained high pH soils. Cyptogram-herb communities are also common on sandy to clay loam soils with increasing cover of *Luzula* sp., bryophytes and crustose lichens (Bliss and Matveyeva, 1992; Bliss, 1997; Bliss and Gold, 1999).

Throughout much of the arctic, plant growth is limited by the availability of N as nutrient inputs via deposition, fixation and weathering are extremely low (Shaver and Chapin, 1980; Jonasson, 1992; Madan et al., 2007; Arens et al., 2008; Callaghan et al., 2010; Gordon et al. 2011). Plants increase their nutrient use efficiency by trapping dead leaves in cushion forms for resorption and translocating nutrients from leaves into woody stems and rhizomes prior to autumn senescence in deciduous species (Maessen et al., 1983; Chapin and Shaver, 1989; Berendse and Jonasson, 1992; Bliss, 1997). Typically in nutrient-poor systems, substantial biomass is allocated to belowground tissue to forage for nutrients in the soil, however polar desert plants produce relatively small root systems compared to the rest of the arctic tundra (Iversen et al. 2015). Root/shoot ratios are typically 0.2 to 0.7 and root elongation is slow

averaging 1.0 to 1.2 cm yr^{-1}, with root growth hampered by low soil temperatures and moisture, stunted and cushion forms as well as soil instability in patterned ground (Bell and Bliss, 1978; Bliss et al., 1984; Bliss et al., 1994; Bliss and Gold, 1999). Root systems consisting of tap roots (*P. radicatum, D. corymbosa* and *S. oppositifolia*) or adventitious roots (*Salix* spp.) are common with shrubs and forbs typically rooting shallower than sedges (Shaver and Cutler, 1979; Miller et al., 1982; Marion et al., 1987; Chapin and Shaver, 1996; Nadelhoffer et al., 1996; McKane et al., 2002; Sullivan et al., 2007). Rooting depths average 15 cm to a maximum of 25 cm (Bell and Bliss, 1978) and the abundance of roots near the surface is largely a result of the thin active layer, frost heave and shallow rhizome networks exploiting inputs by biological soil crust and litter at the soil surface (Jonasson and Sköld, 1983; Bliss et al. 1984; Jonasson and Callaghan, 1992; Bliss, 1997).

Plant roots forage for inorganic forms of N as ammonium (NH_4^+) and nitrate (NO_3^-) that are released for plant uptake upon microbial processing of soil organic matter (SOM). Decomposition, mineralization and nitrification rates are slow due to cold temperatures in the arctic (Hobbie et al., 2000; Giesler et al., 2012; Schaeffer et al., 2013; Wild et al., 2013) and microbial demand for N is very high (Nadelhoffer et al., 1991; Jonasson et al., 1996; Jonasson et al., 2004). Although the seasonality of nutrient availability is not fully understood (Melle et al., 2015), nutrients are often immobilized for the majority of the growing season and can become available in pulses (Schmidt et al., 1999; Tye et al., 2005; Weintraub and Schimel, 2005; Sorensen et al., 2008). The most notable pulse occurs upon microbial dieback during snowmelt at spring (Schmidt and Lipson, 2004; Buckeridge and Grogan. 2010; Larsen et al., 2012) when demand is greatest in plants (Kielland and Chapin, 1992) and water flow supplies mobile nutrients to roots much quicker than diffusion alone (Chapin et al., 1988). To reduce reliance on

microbial processing to supply N, arctic plants take up organic forms of N directly as free amino acids, such as glycine, aspartate, and glutamate (Chapin et al., 1993; Kielland, 1994; Schimel and Chapin, 1996; Nordin et al., 2004). However reliance on organic N and uptake of other forms, particularly peptide- and protein-bound amino acids (Nasholm et al., 2009) remains largely unknown in polar deserts. Although some species show an affinity for certain N forms, for instance the preference of nitrate by *Carex* spp., and organic N and ammonium by sedges, grasses and shrubs (Iversen et al., 2015), most plants take up a mixture of ammonium, nitrate and organic N (Volder et al., 2000; Nordin et al., 2004; Tye et al., 2005). The fraction of each form taken up is related to the species productivity within the plant community and the most productive species use higher amounts of the most abundant forms (McKane et al., 2002). Most arctic plants are able to utilize otherwise unavailable organic N by forming symbiotic relationships with mycorrhizal fungi, with the most frequent associations occurring between ericoid and ecto-mycorrhizae and *Salix, Dryas, Vaccinium* and *Cassiope* spp. (Olsson et al., 2004; Newsham et al., 2009; Fujimara and Egger, 2012). Ericoid and ecto-mycorrhizae form large mycelial networks that release extracellular enzymes to break down complex organic polymers such as proteins and chitins into simple compounds, as well as increase the surface area of the plant's root system to enhance uptake of organic and inorganic nutrients (Michelsen et al., 1996; Hobbie and Hobbie, 2006; Clemmensen et al., 2008; Yano et al., 2010).

2.1.1 Frost Boil Ecosystems

Frost boils are patterned ground features that form from cryoturbation and are common in polar deserts of the Canadian High Arctic (Mackay, 1980; Bliss et al., 1984; Bliss et al., 1994; Anderson and Bliss, 1998; Gold, 1998; Peterson et al., 2003; Ugolini et al., 2006). They occur as small circles, stripes or polygons that range in diameter from <1 to 3 m (Walker et al., 2004).

Despite the many hypotheses on the dominant cryoturbation mechanisms responsible for frost boil formation (Van Vliet-Lanoe, 1991; Vandenberghe, 1998; Kessler and Werner, 2003; Peterson and Kranz, 2003; Ping et al., 2003; Walker et al., 2004; Daanen et al., 2008), they generally agree upon two soil movement regimes: (i) vertical movement upwards at the center and (ii) lateral creeping from the raised center to the edges by frost heave, cryosuction and/or liquefaction of different ice formations trapped within the soil profile as it freezes and thaws (Figure 2.2) (Becher et al., 2013). Movement is cyclical with heave occurring as ice formations freeze and expand in the winter, raising the surface of centers by up to 80 mm yr^{-1} and stony borders by up to 20 mm yr^{-1}, before settling upon thaw in the summer (Hallett, 1998; Overduin and Kane, 2006). Heave is also twice as high at the surface then in subsurface soil at the center of the frost boil (Hallett, 1998). The amount of activity depends largely on water availability, fraction of high water-holding capacity silt particles, thaw depth during the summer and a shallow permafrost table that impedes drainage (Bockheim and Tarnocia, 1998; Walker et al., 2004; Overduin and Kane, 2006; Michaelson et al., 2008; Klaus et al., 2013). These two movement regimes organize frost boils into "islands of fines" of exposed, raised centers of silty-clay mineral soil with coarse gravel and rocks concentrated in depressional outer rims (Ugolini et al., 2006). The inter-boil region surrounding the rim varies in cover and can range from a barren mixture of coarse-material and large stones to vegetated areas of fine soil (Walker et al., 2008). Frost boils are defined as sorted if a border of rocks surrounds the rim or non-sorted if a border is lacking (Becher et al., 2013).

Cryoturbation disturbances result in sharp habitat differentiation of plant communities within frost boil ecosystems. Disturbances increase soil temperatures, thaw depth and availability of new soil substrates to favour nutrient cycling and inputs in frost boils over surrounding inter-

boil regions (Walker et al., 2004; Michaelson et al., 2008; Walker et al., 2008; Callaghan et al., 2010; Kelley et al., 2012). Plant productivity as well as seedling recruitment is greater in frost boils and most plants are situated near the outer rims where stability is greatest, particularly large mats of heath vegetation such as *S. arctica* and *C. tetragona* (Bliss, 1997; Cannone et al., 2004; Sutton et al., 2006). The abundance of plants further from the center also coincides with an increase in A horizon thickness, nutrients, moisture and biological soil crust cover (Bliss et al., 1994; Anderson and Bliss, 1998; Bliss and Gold, 1999; Haugland, 2006; Makoto and Klaminder, 2012). The development of a biological soil crust is one of the most important factors controlling plant establishment in polar deserts as it holds in moisture, increases temperature and fixes large amounts of N to favour plant survival and offer "safe sites" for rapid seedling establishment (Gold and Bliss, 1995; Gold, 1998; Bliss and Gold, 1999; Dickson, 2000; Sutton et al., 2006;

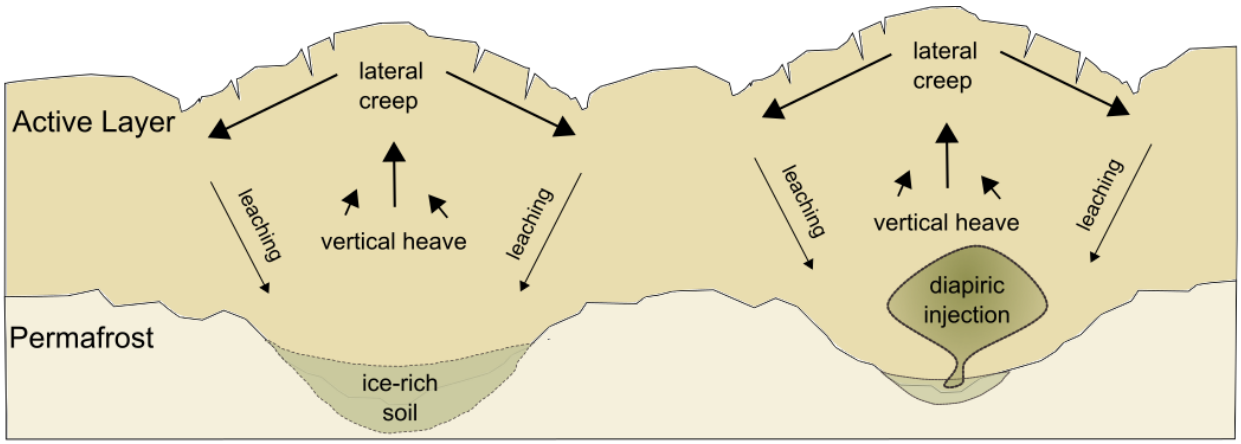

Figure 2.2. A conceptual diagram of soil movement regimes and diapir formation in frost boils. Solid arrows indicate the direction of soil movement of the major (thick line) cryoturbation mechanisms that occur during annual freeze/thaw cycles and result in frost boil formation. The profile of the permafrost table is inversely related to the surface in frost boils such that the permafrost table is deepest in the center and shallowest at the edges and a concave bowl develops below the frost boil center. The processes of leaching and accumulation of ice-rich soil in the concave bowl of the permafrost table over successive years (frost boil on left) can create a diapiric injection (frost boil on right). Adapted from Walker et al. (2004).

Stewart et al., 2011a). Seeds are often found in higher abundance in centers, however their establishment is often impeded by drying of surface soils, surface settling and seedling upheaval following frost heave (Anderson and Bliss, 1998; Kelley and Epstein, 2009). Although active barren centers pose a difficult micro-habitat for establishment, forbs and graminoids that have elastic, shallow and/or small diameter roots, and small stress-tolerant individuals and basophiles such as *Cardamine bellidifolia, D. integrifolia, Juncus biglumis* and *Saxifraga* spp. are able to grow (Jonasson and Sköld, 1983; Jonasson and Callaghan, 1992; Anderson and Bliss, 1998; Cannone et al., 2004). If frost boils become inactive for longer time periods, plants (often shrubs) can encroach in from the rims to cover and further stabilize the frost boil by decreasing the heat flux and thaw depth in the summer (Haugland, 2006; Kade and Walker, 2008; Walker et al., 2008; Makoto and Klaminder, 2012).

Vertical mixing and lateral soil creep across the upheaved centers downslope into outer rims creates a heterogeneous distribution of plant-available nutrients throughout frost boils. Soil organic carbon and N (mainly ammonium and dissolved organic N (DON)) typically increase outwards from the center coinciding with an increase in moisture, biological soil crust cover, microbial biomass and activity, and decrease in frost heave (Mueller et al., 1999; Walker et al., 2004; Kaiser et al., 2005; Sorenson et al., 2006; Michaelson et al. 2008; Stewart et al. 2011a; Michaelson et al., 2012). In wetter margins, decomposition and turnover of SOM occurs relatively quickly, however increased mineralisation is often nullified by even higher immobilisation rates that can limit the availability of nutrients to plants (Kaiser et al., 2005). Organic-rich materials from litter and biological fixation by crust accumulate at the surface and can be transported downwards through leaching and burial during horizon mixing by cryoturbation (Sorenson et al., 2006; Phillips, 2011; Palmtag et al., 2015). Within the active layer

– SOC commonly increases with depth and total N decreases – usually due to ammonium (the most abundant form of available N) remaining at the surface after being fixed by cyanobacteria in surface crust while small amounts of mobile nitrate concentrate with moisture in deeper horizons (Kaiser et al., 2005; Horwarth et al., 2008; Phillips, 2011; Brummell et al., 2012). In non-sorted circles at Spitsbergen, Norway, Boike et al. (2008) mapped the lateral and vertical distribution of SOC and N and found low levels throughout the majority of the frost boil from center to edge and surface to deeper soil, except for a single highly concentrated patch deep below the center. Cryoturbation processes can redistribute SOC not only from surface horizons but also – and perhaps more importantly in unproductive High Arctic ecosystems – from underlying permafrost (Ugolini et al., 2006; Keuper et al., 2012; Ping et al., 2015).

2.1.2 Diapirism

During summer thaw, snowmelt and dissolved organics leach down through the soil profile and pool below the center of the frost boil in the concave bowl of the permafrost table (Peterson et al., 2003; Cannone et al., 2004; Ping et al., 2015). In some frost boils, a large pocket of low-density ice-rich soil develops in the lowermost part of the active layer and uppermost permafrost over multiple years of accumulation (Figure 2.2, left). Eventually as this material becomes lighter than the overlying soil it creates an unstable soil density profile and during periods of maximum thaw in late summer, the dense overburden sinks to cause an often viscous upwards flow of the underlying material (Figure 2.2, right). The upward flow forms a diapiric injection, up to several decimetres high and wide, of permafrost material in the active layer below the center of the frost boil that resembles a mushroom-shaped plume (Swanson et al., 1999). As the soil freezes during winter, vertical heave can then transport the diapiric injection higher, often within 25 cm of the surface in polar deserts, as shallow permafrost tables occurring

at 50 cm depth are common (Bliss, 1997). Reports of diapiric injections have been detailed in various patterned ground features of low arctic tundra (Mackay, 1980; Van Vliet-Lanoë, 1991; Kling, 1996; Swanson et al., 1999) and beyond a single account of diapiric "plugs" by Ugolini et al. (2006) it is relatively unknown how widespread they are across polar deserts of the high arctic. Furthermore as temperatures, permafrost degradation, moisture (in the form of snowfall) and cryogenic activity are predicted to increase over the next century (IPCC, 2013), the impacts on diapir formation and processes are uncertain but likely to become more prevalent in polar desert regions (Callaghan et al., 2005; Klaus et al., 2013; Nowinski et al., 2013).

Once in the active layer, temperature and moisture increase in the diapiric injection, and the previously unavailable SOC originating from the permafrost is predicted to fuel microbes and form an overlying horizon enriched in SOM. However, the amount of microbial activity and enrichment with SOC and other nutrients – particularly N – in the diapir horizon, and its potential as a source of plant-available nutrients has yet to be investigated. Other work across the arctic suggests microbial decomposition of permafrost-origin carbon would occur at deeper depth but at a slower rate than inputs at the surface (Mu et al. 2014; Jansson and Tas, 2014; Wild et al. 2014). In cryoturbated topsoils that had been redistributed deeper in the soil profile, bacterial abundances were as high as at the surface - however lower temperatures and oxygen caused a decrease in fungal abundance (Gittel et al., 2014) that ultimately slowed decomposition by lowering the capacity for communities to break down proteins (Wild et al., 2013). Shallow diapir horizons coupled with a warmer environment in the frost boil center where thaw depths reach deepest (Ugolini et al., 2006; Boike et al., 2008; Daanen et al., 2008), are likely to promote temperatures sufficient for adequate microbial activity to enrich the horizon in SOM and increase plant-available nutrients such as N if immobilization rates stay low (Biasi et al., 2005; Keuper et

al., 2012; Jansson and Tas, 2014). Furthermore, a feedback loop is likely to be created if plants are able to access diapir nutrients, whereby plant root exudates and turnover stimulate microbial activity to in turn increase the amount of nutrients available for plants (Farrar et al., 2003; Iversen et al., 2015).

The diapir horizon represents an ecologically significant source of nutrients that could favour polar desert plant growth and survival in an otherwise extremely limited and nutrient-poor ecosystem. When encountering a nutrient-rich zone, plants alter the growth and uptake capacities of their root system and preferentially place roots into the patch over the surrounding soil, particularly if patch contrast is high to background fertility (Robinson, 1996; Hodge et al., 1999; Hutchings et al., 2003; Hodge, 2004; Cahill et al., 2011). In larger, predictable patches, plants commonly exploit the patch morphologically by increasing root length, root production, lateral branching, residency time and/or reducing root mortality (Gross et al., 1993; Hodge, 2004; McNickle et al., 2009). In temporally unpredictable or infrequent patches of smaller size plants also respond physiologically by altering the rate of nutrient absorption from the soil solution and increase individual roots' uptake capacity or ion affinity in response to a pulse of nutrients (Jackson et al., 1990; Hodge, 2004; Makita et al., 2010; Mou et al., 2013). Although arctic plants are limited by the short, cold growing season, they have a high tolerance for deep, cold soils and maintain growth and high uptake capacities at low temperatures (Chapin, 1974; Atkin and Cummins, 1994; Kielland and Chapin, 1994; Bilbrough et al., 2000), with the ability of certain species to grow directly onto permafrost without damage (Edwards and Jeffries, 2010). Although polar desert root systems are typically slow-growing and shallow (averaging 15 cm), their ability to develop and function in deep soil would predict plants to favourably proliferate roots into and exploit large, predictable patches of diapir nutrients. Deeply rooted species such as *S. arctica*, *S.*

oppositifolia, and *P. radicatum* would likely gain a greater advantage in frost boil ecosystems by locating diapir horizons more rapidly upon movement into the active layer - particularly so since peak root production (Bell and Bliss, 1978) and nutrient uptake (Chapin,1980; Kielland and Chapin, 1992) coincides with peak soil movement (Mackay, 1980; Swanson et al., 1999) at the end of the growing season, and uptake often continues well after aboveground tissue has senesced (Chapin and Bloom, 1976), even extending into winter in *Salix* spp. (Andresen and Michelsen, 2005).

2.2 Tracing Nitrogen in the Soil-Plant System

Stable isotopes occur naturally in the environment and are used to trace the flow of nutrients and unravel processes in the soil-plant system (Bahn et al., 2012). Natural materials are composed of elements with stable isotopes that differ in mass and the concentration of heavy and light stable isotopes in a nutrient source can be used as a "fingerprint" or signature to determine the source's fate in the environment using the natural abundance method. The natural abundance method measures exceedingly small differences in ratios of heavy to light stable isotope pairs and is expressed as a delta (δ) in parts per thousand (‰). A nutrient source is followed from the soil into the plant by measuring distinct δ signatures of every nutrient source in the soil and determining which source matches the δ value of the plant (Dawson et al., 2002). However, biological and chemical reactions during nutrient cycling change the partitioning of heavy and light isotopes (termed fractionation) and can cause the signature of the nutrient source to be lost (Sulzman, 2007). Fractionation factors for many transformations often cause predictable changes in δ values and the relative magnitude of changes during cycling can instead be used as an integrator to shed light on the major processes occurring in the system (Robinson, 2001). An alternate tracer approach that is unaffected by fractionation processes is the enriched isotope

method. The enriched isotope method traces the fate of a nutrient by adding an isotopically-enriched nutrient source at elevated levels well outside the natural isotopic range directly into a soil source of interest and measuring the concentration in plant tissue following an incubation period to determine if the nutrient has been taken up directly from the labelled source (Dawson et al., 2002).

Nitrogen is composed of two stable isotopes (^{14}N and ^{15}N) and is traced through the environment using the isotope ratio $^{15}N/^{14}N$ (referred to as ^{15}N) (Bedard-Haughn et al., 2003). Enriched isotope and to a lesser extent natural abundance methods have been used extensively to trace N in the soil-plant system, with many applications in the arctic tundra (Amundson et al., 2003; Craine et al., 2009; Templer et al., 2012). The use of ^{15}N-enriched tracers was originally developed to easily detect N flows through sources and sinks in the soil and differentiate between external and internal N sources in the plant (Dawson et al., 2002). Another common application is applying mixtures of ^{15}N-enriched organic (e.g. ^{15}N-glycine) and inorganic (e.g. $^{15}NH_4^+$, $^{15}NO_3^-$ and $^{15}NH_4{}^{15}NO_3$) nutrients to the soil and determining temporal variations in nutrient uptake (Andresen and Michelsen, 2005; Sorensen et al., 2008; Edwards et al., 2010) or N source preference in plant species (McKane et al., 2002; Nordin et al., 2004; Clemmensen et al., 2008). However, application of enriched nutrients can lead to fertilization effects, particularly in N-limited systems (Bedard-Haughn et al., 2003), and the natural abundance method provides a better understanding of N processes and cycling patterns at natural levels without any external alterations. Soil is typically enriched in $\delta^{15}N$ relative to plants, and the two are linked through a series of transformations that occur as N is processed through different pools in the system (Figure 2.3). Soil N turnover is slow and $\delta^{15}N$ values often reflect longer term processes, such as SOM turnover where higher soil $\delta^{15}N$ can indicate an increase in nitrification and denitrification

rates (Chapin, 1996). Plant $\delta^{15}N$ is more indicative of processes occurring in the short-term, such as N availability of different N pools in the soil, however $\delta^{15}N$ values are often confounded by internal fractionations or mycorrhizal transfers and must be interpreted with care (Marshall et al., 2007). In N-limited arctic ecosystems, $\delta^{15}N$ values are often depleted relative to low latitude ecosystems where higher N supply, availability and transformation rates gradually enrich the system in ^{15}N (Makarov, 2009; Craine et al., 2015).

2.2.1 Measuring ^{15}N

The natural abundance isotopic composition of N is defined as:

$$\delta^{15}N = \left(\frac{R(^{15}N/^{14}N)_{sample}}{R(^{15}N/^{14}N)_{standard}} - 1 \right) 1000 \qquad \text{(Eq. 2.1)}$$

where R_{sample} and $R_{standard}$ refer to the $^{15}N/^{14}N$ ratio measured in the sample and standard of atmospheric N_2, respectively and values are reported in parts per thousand (‰) (Chalk et al., 2015). A δ value that is positive indicates an enrichment in the concentration of ^{15}N isotope, or depletion when negative, relative to atmospheric N_2. During N transformations, isotopic differences between the source and product can occur due to equilibrium and kinetic mechanisms during the chemical reaction, termed fractionation. The amount of fractionation is often quantified by the degree to which a process avoids production of the heavy ^{15}N isotope, termed discrimination ($\delta^{15}N_{substrate} - \delta^{15}N_{product}$) (Sulzman, 2007). In enriched isotope experiments, the nutrient source added into the environment is labelled with an isotopic composition that is significantly higher than natural values (Dawson et al., 2002). The isotopic composition of material sampled from isotopically-enriched systems is defined as:

$$\text{atom\%}^{15}N = \frac{\text{amount of } ^{15}N}{\text{amount of } ^{14}N + ^{15}N} = \left(\frac{R(^{15}N/^{14}N)_{sample}}{R(^{15}N/^{14}N)_{sample} + 1} \right) 100 \qquad \text{(Eq. 2.2)}$$

where the values are reported in percent (%) (Chalk et al., 2015). N isotope ratios are measured by reducing N in samples to N_2 by combustion in a continuous-flow isotope ratio mass spectrometer (Sulzman, 2007).

2.2.1.1 Factors Influencing $\delta^{15}N$

Many different inputs and processes affect soil and plant $\delta^{15}N$ (Figure 2.3), however changes in the isotopic ratio are directed by the same factors and reflect the source, fractionations, gains and losses, and mixing of N pools in the soil-plant system. The $\delta^{15}N$ of an N source (e.g. soil nutrient patch) and N sink (e.g. plant) in a system abide by four rules: (i) fractionations associated with the transformation of an N pool are flexible (e.g. conversion of soil NH_4^+ to NO_3^- during nitrification) and substrate (e.g. NH_4^+) and product (e.g. NO_3^-) pools change along a known distribution, (ii) the total $\delta^{15}N$ of a system changes only when a source enters or leaves, (iii) when a pool divides, the $\delta^{15}N$ of any resulting pools are equal to the original if fractionation does not occur, and (iv) the $\delta^{15}N$ of a source or sink is the mass-weighted mean of the $\delta^{15}N$ of each pool in the source or sink (Robinson, 2001). Observing the environmental patterns in $\delta^{15}N$ of N pools and transformations using these rules can reveal nutritional links between soil and plants in an ecosystem.

The $\delta^{15}N$ of a soil nutrient source is largely dependent on several fractionation factors that occur during N transformations associated with SOM processing. In most reactions, use of the light ^{14}N isotope is favoured and microbial decomposition processes such as enzymatic hydrolysis, mineralization and nitrification lead to the creation of ^{15}N-depleted inorganic N forms in the soil and volatilization of ^{14}N into the atmosphere as NH_3, N_2, NO and N_2O (Craine et al., 2015). When these ^{15}N-depleted forms are assimilated by plants or microbes, leached or lost to the atmosphere, the residual soil $\delta^{15}N$ will often increase (Hobbie and Ouimette, 2009).

Plant-available pools are generally assumed to decrease in $\delta^{15}N$ values from DON > NH_4^+ > NO_3^-, although these relationships are often disrupted by competing reactions (Evans, 2007). Individual organic compounds (e.g. amino acids, proteins) have a wide variation in $\delta^{15}N$ and

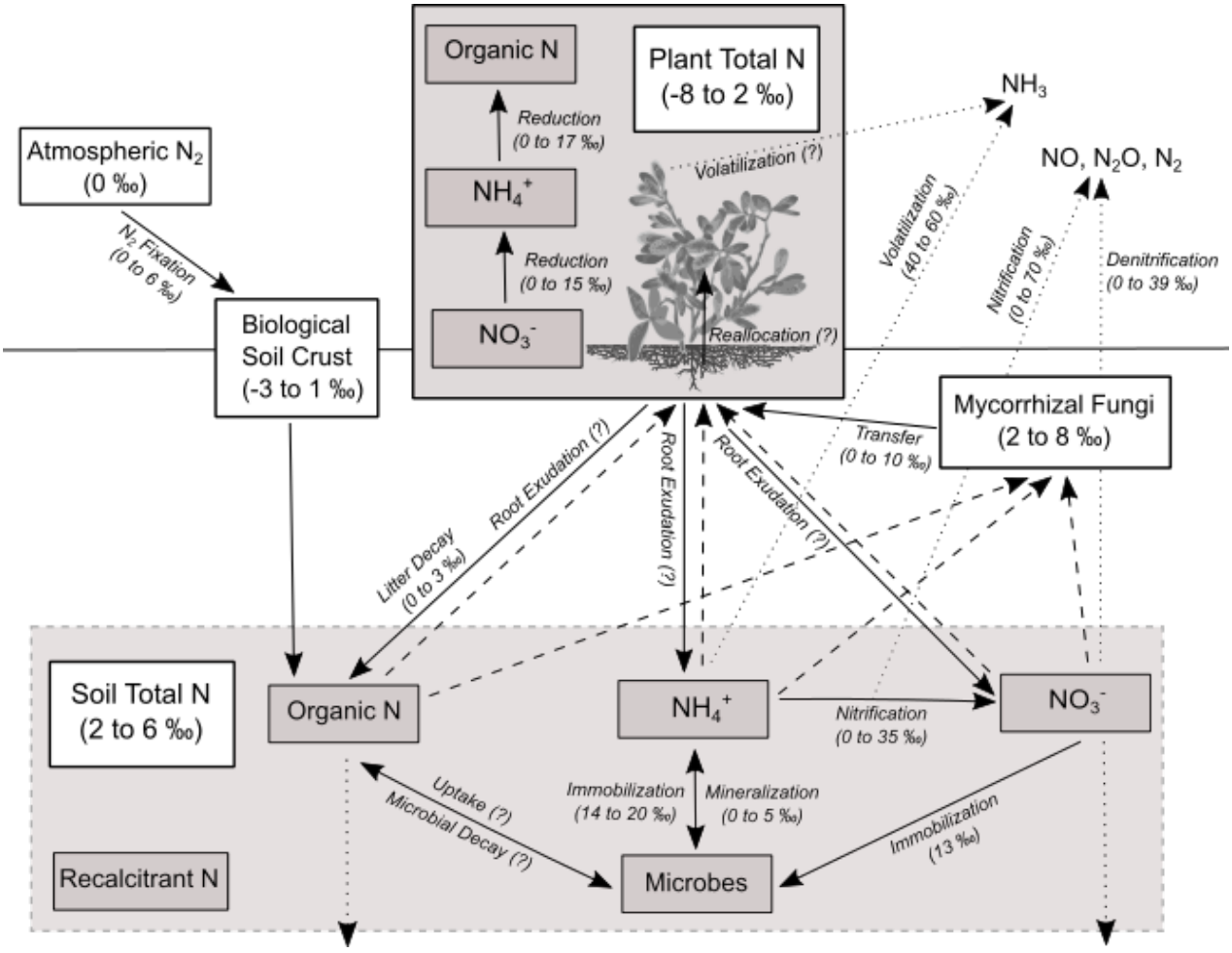

Figure 2.3. A conceptual diagram of the major nitrogen isotopic pools and transformations in the soil-plant system, adapted from Dawson et al. (2002). The average range of $\delta^{15}N$ in pools (boxes) and discrimination values of transformations (arrows) that enrich of deplete pools in ^{15}N upon fractionation are reported. In N-limited systems, negligible fractionation can occur during mycorrhizal and plant root uptake (dashed arrows) and leaching and gaseous losses can transport N out of the system (dotted lines). Discrimination values of many transformations require further study (?) and some transformations are not reported (e.g. transamination of amino acids in organic N pools). Values sourced from Evans (2007), Hobbie and Hobbie (2008), Makarov et al. (2008), Makarov (2009), Hobbie and Högberg (2012), and Craine et al. (2015).

transamination or hydrolysis reactions can cause enrichments or depletions in DON relative to inorganic forms (Michelsen et al., 1998; Yano et al., 2010). In N-limited systems, NH_4^+ is typically enriched over NO_3^- (by up to 6 ‰) unless substantial denitrification occurs and enriches NO_3^- to levels above NH_4^+ (Evans, 2007; Hobbie and Högberg, 2012). Total N in the soil is typically equal or ^{15}N-enriched when compared to DON and inorganic forms, respectively, however considerable variation also exists in these relationships (Craine et al., 2015). The $\delta^{15}N$ of total N and NH_4^+ are similar when nitrification is low, but $\delta^{15}N$- NH_4^+ is positively correlated to nitrification rates and can surpass total N as rates increase (Makarov et al., 2008; Mayor et al., 2012). Soil microbial biomass $\delta^{15}N$ is often more enriched than both DON and total N, and can affect these pools upon production of ^{15}N-depleted exudates as well as during microbial turnover and decay of the ^{15}N-enriched biomass, an important factor in slow turnover environments such as the arctic (Craine et al., 2015). The rapid turnover of NH_4^+, NO_3^-, amino acids and microbial N pools can cause large variations over short-time periods (up to 20‰ in individual pools) (Evans, 2001) and continuous sampling over a longer period is often necessary to characterize the isotopic compositions of available N (Hobbie and Ouimette, 2009). Although the $\delta^{15}N$ of total N reflects both the recalcitrant (turnover time of hundreds of years) and labile (turnover time of days to months) pools, the signature is usually dominated by the former as it makes up a larger portion of N in the soil and in many cases may not represent the fraction available to the plant (Högberg, 1997).

The $\delta^{15}N$ signature in the soil profile varies and depends largely on the frequency and composition of N inputs and degree of microbial processing. Plants often have considerably lower $\delta^{15}N$ than soil and litterfall from ^{15}N-depleted plant foliage and root inputs cause lower soil $\delta^{15}N$ at the surface (Hobbie et al., 2000; Rhoades et al., 2008). While the $\delta^{15}N$ of litter generally

increases over time, the isotopic composition can increase or decrease during initial decomposition stages depending on the environmental controls of decay, the sequence of compounds that are degraded and activity of the microbial biomass (Craine et al., 2015). Eventually as residence time increases, SOM begins to reflect the signatures of decomposers rather than initial litter inputs (Kramer et al., 2003; Evans et al., 2007). Biologically-fixed N derived from the soil crust is ^{15}N-depleted and can further lower surface δ^{15}N in areas of high cover (Stewart et al., 2011b; Skrzypek et al., 2015). Deeper in the soil, inputs from ^{15}N-depleted root (that is often ^{15}N-enriched relative to foliage) and ^{15}N-enriched mycorrhizal fungi accumulate (Högberg, 1997; Wallander et al., 2009) and enrich the soil in ^{15}N relative to the surface (Hobbie and Ouimette, 2009). Fungal residues (particularly ecto-mycorrhizal) are long-lived with a carbon turnover time of up to 5 years (Treseder et al., 2004). Decomposition occurs more slowly than in plant roots, and fungal residues often accumulate in greater amounts relative to other residues in the soil (Wallander et al., 2009). The enrichment in deeper soil horizons is also attributed to the high δ^{15}N value of recalcitrant N and humified OM that result from heavy microbial processing over time (Evans, 2007). Furthermore, the mixing of soil N sources (e.g. cryoturbation processes) and leaching of surface materials can create a δ^{15}N value that is intermediate between sources (Robinson, 2001).

Plants contain a mixture of stored and recently derived N and early assumptions predicted δ^{15}N to largely reflect the major nutritional sources taken up from the soil (Högberg, 1997). It is now clear that plant δ^{15}N is much more complex and influenced not only by the soil source, but by several additional factors and interactions that are often species specific. Although uptake of N into the root is considered to cause negligible fractionation, assimilation of inorganic N into organic compounds within the plant can cause high fractionation values that are dependent upon

the N concentration at the root surface (Craine et al., 2015). In most natural ecosystems, plants take up a the majority of available N in the rhizosphere and fractionation values are negligible but can increase when smaller portions of N are taken up from the soil as N concentration increases and/or nutrient use efficiency decreases (Marshall et al., 2007). In N-limited systems, such as high latitude polar deserts, there is a greater potential for plant $\delta^{15}N$ to reflect soil N sources and increased mineralization and nitrification rates can lead to higher $\delta^{15}N$ values in the plant (Makarov, 2009). Rooting depths also affect plant $\delta^{15}N$ as shallow-rooted species derive more N from ^{15}N-depleted surface horizons and have lower $\delta^{15}N$ than species with deeper rooting systems (Nadelhoffer et al., 1996). One of the largest influences on plant $\delta^{15}N$ is the reliance on mycorrhizal symbionts to supply a substantial portion of plant N (up to 60% in arctic tundra) (Yano et al., 2010). Plant $\delta^{15}N$ is depleted upon transfer of N as mycorrhizal fungi preferentially retain ^{15}N (Hobbie et al., 2000) and the degree of fractionation depends upon the type of symbiont. Plant species that associate with arbuscular, ericoid and ecto-mycorrhiza (such as *S. arctica*), are depleted by 2, 3 and 6‰, respectively relative to non-mycorrhizal species (Craine et al., 2009).

Plant $\delta^{15}N$ is commonly represented by $\delta^{15}N$ of the foliage as it contains the greatest portion of N in the plant (Högberg, 1997). However, considerable isotopic variation can occur throughout an individual plant's leaf, stem and root (Craine et al., 2015). Mycorrhizal associations often cause the greatest intra-plant variation and fractionation is highest in species with ectomycorrhizal symbioses, reaching up to a 4‰ depletion in roots relative to leaves (Michelsen et al., 1998; Hobbie and Colpaert, 2003). Variation of signatures in above- and belowground tissues are also caused by reallocation of N in tissues of deciduous species (Kolbl and Evans, 2002) and NH_3 fluxes from foliage whereby volatilization can increase $\delta^{15}N$ and

uptake can deplete $\delta^{15}N$ in leaf tissue (Johnson and Berry, 2013). Within plant cells, enrichment of inorganic N pools and depletion of organic N pools can occur due to discrimination against ^{15}N by enzymes responsible for the reduction of NO_3^- and NH_4^+ into organic compounds (e.g. nitrate reductase and glutamine synthetase, respectively) after uptake into the plant (Craine et al., 2015). Increasing uptake of NO_3^- can result in greater intra-plant variation as it is involved in an additional reduction step (reduction into NH_4^+ by nitrate reductase) and can be assimilated anywhere in the plant, unlike NH_4^+ assimilation that is limited within the root (Evans, 2001).

3. DIAPIRISM ALTERS THE NITROGEN FORAGING STRATEGY OF *SALIX ARCTICA* IN FROST BOILS OF A HIGH ARCTIC POLAR DESERT

3.1 Abstract

In polar desert ecosystems, diapiric intrusions of re-distributed SOC occur within frost boils - circular islands of fine soil that are associated with increasing plant productivity. Plant growth in polar deserts is strongly limited by N availability and the diapir horizon has the potential to provide an essential N source for plant survival that has yet to be studied. To determine if diapir horizons provided an enriched plant-available source of N, uptake by *Salix arctica* was characterized using ^{15}N natural abundance and enriched isotope methods at a polar semi-desert site in the Canadian High Arctic. In the absence of a diapir horizon, *S. arctica* took up N from the largest soil N source in the frost boil located at the surface. However as diapir horizons became available, *S. arctica* altered its foraging strategy by limiting uptake at the surface and increasing from the sub-surface diapir patch. Plants appeared to selectively place roots in diapir nutrient zones and increased ^{15}N-enriched isotope uptake by 2.5 fold over other sub-surface N rich patches. These findings suggest the diapir horizon is important for plant production in frost boils and increasing diapir prevalence has the potential to increase plant growth, particularly of deciduous shrub communities, in these barren ecosystems.

3.2 Introduction

The majority of ice-free land in the arctic is a barren desert of scattered plants, covering less than 5 to 20% of the soil surface (Bliss and Gold, 1999). Nitrogen is extremely limiting in the cold, dry soil environment and SOM inputs primarily occur at the surface from scarce litterfall and N_2 fixation by cyanobacteria (*Nostoc* spp.) present in a thin layer of biological soil crust (Bliss et al., 1984; Dickson, 2000; Stewart et al., 2011a). Surface organic material is frequently redistributed deeper in the soil profile by leaching or cryoturbation (Bockheim, 2007; Horwarth et al., 2008; Ping et al., 2008b; Becher et al., 2013). Much of the remaining SOM is frozen in permafrost (Michaelson et al., 2008; Tarnocai et al., 2009; Palmtag et al., 2015) and highly vulnerable to the increases in temperature (2 to 8°C), and associated permafrost degradation predicted for the Canadian Arctic Archipelago and Greenland by the end of the 21st century (IPCC, 2013).

Patterning the ground of many polar desert landscapes is a patchwork of frost boils – circles of fine soil associated with increasing cover of plants (Walker et al., 2004; Ugolini et al., 2006). Frost boils are formed from the churning action of annual freeze-thaw cycles that upheave the centers and limit plant establishment to the stable outer rims (Cannone et al., 2004; Walker et al., 2008; Michaelson et al., 2012). Within some frost boils, cryoturbation processes redistribute material from the underlying permafrost to form an enriched horizon below the soil surface (Swanson et al., 1999; Ugolini et al., 2006; Horwarth et al., 2008). A diapiric injection develops when moisture pools in a concave depression below the frost boil center and low-density ice-rich soil develops in the uppermost permafrost. As the overlying high-density material in the active layer sinks, the permafrost material flows upwards into the active layer and can be transported often within 25 cm of the soil surface by vertical heave (Swanson et al., 1999). Temperature and

moisture increases in the active layer unlock previously unavailable SOC that is predicted to fuel biological activity and form a Bhy horizon that is enriched in N (Jannson and Tas, 2014; Mu et al., 2014; Wild et al., 2014).

Plants in polar deserts typically produce smaller and shallower root systems (Bell and Bliss, 1978) than in other tundra vegetation types (Iversen et al., 2015). Stunted growth and cushion forms typical of many arctic species along with limited soil moisture and soil instability due to frost heave are often the main causes of limited root production in polar deserts (Jonasson and Sköld, 1983; Bliss et al., 1984; Jonasson and Callaghan, 1992). However, arctic species have a high tolerance for cold soils with the capacity to maintain nutrient uptake capacities and growth rates in these conditions (Atkin and Cummins, 1994; Bilbrough et al., 2000; Volder et al., 2000; Larsen et al., 2012), and can even extend their roots directly into permafrost (Edwards and Jeffries, 2010). It is well established that many plants forage for nutrients in heterogeneous soils by selectively proliferating roots into nutrient-rich patches (Robinson, 1996; Hutchings et al., 2003; Hodge, 2004; McNickle et al., 2009; Cahill et al., 2011). The diapir horizon is a large nutrient-rich patch in an otherwise nutrient poor environment, however it is unknown how arctic plant species respond to diapirs. The ability of arctic species to grow and function in deep, cold soils typical of diapir horizons suggests plants, particularly deep-rooted (e.g. *S. arctica*, *Papaver radicatum*) or elastic-rooted species that can withstand frost heave (e.g. *Saxifraga* spp.), would proliferate roots into and exploit these patches if they were enriched in N. Furthermore, if plants did utilize diapir horizons, those roots may create a feedback loop whereby exudates and turnover of roots and mycorrhizal hyphae stimulate microbial decomposers to further increase plant-available N (Farrar et al. 2003, Langley and Hungate, 2003; Sullivan et al., 2007; Sloan et al., 2013).

The enrichment of N in diapir horizons and potential for these diapirs to act as a critical N source for plants in extreme polar desert environments has yet to be investigated. The flow of N through the soil and into the plant root upon uptake can be traced using naturally-occurring $\delta^{15}N$ signatures and external additions of ^{15}N-enriched isotopic labels (Dawson et al., 2002; Bedard-Haughn et al., 2003; Evans, 2007). $\delta^{15}N$ signatures of soil sources can further be used to characterize the origins of inputs (e.g. biologically-derived N versus mineral N) as well as SOM processing rates (e.g. ^{15}N enrichments and depletions can indicate changes in mineralization, nitrification and denitrification rates, and accumulation of recalcitrant material) (Makarov, 2009; Craine et al., 2015). Substantial mixing of nutrient sources or internal fractionations within the plant can limit the inferences possible from natural abundance methods (Evans, 2001; Robinson, 2001; Kalcsits et al., 2014). Recovery of ^{15}N-enriched tracers in plant tissues can be low in arctic environments (often less than 10%) due to microbial immobilization of the majority of the label but the addition of highly-enriched dual-N nutrients (e.g. 98 atom% $^{15}NH_4{}^{15}NO_3$) can increase recovery and provide a direct tracer into the plant that is unaffected by fractionation processes (Templer et al., 2012).

In this study the natural abundance of $\delta^{15}N$ and addition of ^{15}N-enriched nutrients were used to characterize the N interactions between diapir horizons and *S. arctica*, the dominant vascular plant species, in a semi-polar desert of the Canadian High Arctic. The objectives were to determine whether (1) diapir horizons were enriched in N, and (2) accessible by *S. arctica* roots and an important N source for survival in frost boil ecosystems of the polar desert landscape.

3.3 Materials and Methods

3.3.1 Field Site

Field work took place on Dome site (78°51'N, 76°06'W), a mountain plateau located five km southwest of Alexandra Fiord, Ellesmere Island, NU during July and August of 2013. The site is characterized by two distinctive soil types formed from granitic and dolomitic parent material. The granitic material (650 m.a.s.l) is located on a gentle gradient (2 to 5°) upslope of the dolomitic material (500 m.a.s.l) to the east and downslope of a glacier (750 m.a.s.l.) to the northwest. There is a stark contrast in pH across the site with low pH in granitic material (pH 5.5) increasing into dolomitic material (pH 7.9) (Bliss et al., 1994). Patterned ground features are common on the soil surface occurring as frost boils ranging in expression from small sorted or non-sorted circles and stripes that vary in development (Figure 3.1). The soil is dominated by Regosolic Turbic Cryosols with limited horizon development, very low organic matter and low levels of plant-available N and P (Brummel et al., 2012). Cryptogamic crusts of bryophytes (e.g. *Pogonatum denatum*), lichens (e.g. *Catallaria subnegans* and *Cladonia* spp.) and cyanobacteria (e.g. *Nostoc commune*) establish on moist surfaces (Bliss et al., 1994).

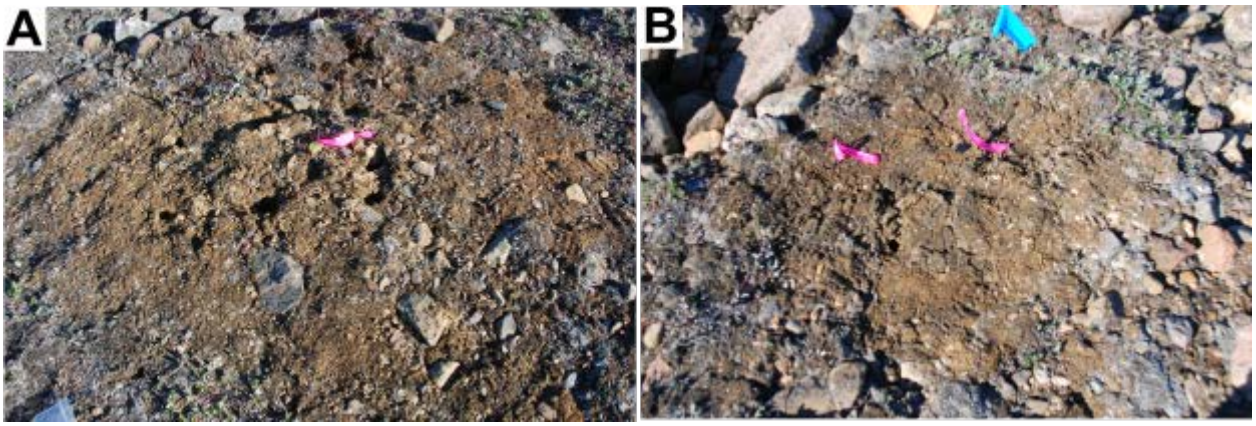

Figure 3.1. Frost boils varied in development across Dome site and occurred as weakly developed non-sorted circles with poor sorting of coarse material to the edge (A) to highly developed sorted circles with a centre of fines (B).

3.3.2 Identifying Diapir Frost Boils

Twelve blocks on granitic and three blocks on dolomitic parent material, each of approximately 50 m by 50 m, were established to ensure frost boils representative of the full range of frost boil features, slope, water availability and microclimate across the landscape were selected. Within each block, the first five frost boils encountered on four perpendicular transects originating from the center of the block (n=20) were sampled with a field portable visible and near-infrared spectrometer (vis-NIR) (Veris Technologies, Salina, KS) to determine whether diapir horizons were present. The spectra was calibrated to predict SOC using a partial least squares regression model (Guy et al., 2015) and spectral profiles were taken in situ by inserting a soil probe into the center of the frost boil and sampling at two cm increments from the surface to the top of the permafrost layer (ca. 40 cm). A diapir frost boil was identified by an enriched SOC peak that increased by ≥ 3 g SOC kg^{-1} dry soil below the soil surface (Figure 3.2B) and a control frost boil by the absence of a peak (Figure 3.2A).

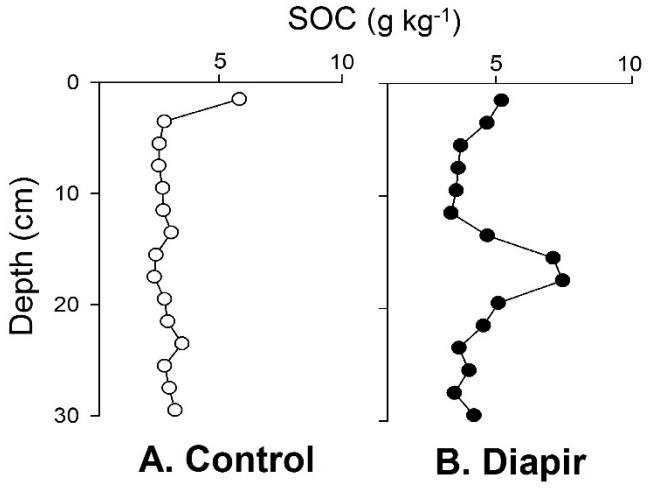

Figure 3.2. Example of vis-NIR spectra profiles of the active layer in a control (Panel A) and diapir (Panel B) frost boil. In the diapir frost boil, a diapir horizon occurs between 13.5 and 19.5 cm in depth with the peak in SOC occurring at 17.5 cm depth.

3.3.3 Natural Abundance Isotope Experiment

One diapir and one control frost boil in each block on granitic parent material (n=24) was sampled for soil cores and *S. arctica* tissue between July 26th and August 3rd. Sets of leaf, aboveground stem and belowground stem/root tissue were collected from three individual *S. arctica* plants located on each frost boil (n=9). Soil samples were collected from pits in 5 cm increments from 0 to 35 cm depth at the center of each frost boil. Soil samples were separated into surface, high, medium and low spectra categories (n=4) using the vis-NIR spectra profile. High spectra were defined for each frost boil from the peak of the SOC curve in a diapir horizon in a diapir frost boil (e.g. 19.5 cm in Figure 3.2B) or from a relatively high spectra in a control frost boil (e.g. 25.5 cm in Figure 3.2A). Medium and low spectra categories were taken in decreasing SOC content, respectively, from the remaining soil profile.

Values for $\delta^{15}N$, total N and total C of soil and vegetation samples were analyzed on a thermal conversion/elemental analyzer isotope ratio mass spectrometer (TC/EA-IRMS ThermoFinnigan Delta Plus). The N isotopic composition was expressed as a delta (δ) in parts per thousand (‰) and defined as:

$$\delta^{15}N = \left(\frac{R(^{15}N/^{14}N)_{sample}}{R(^{15}N/^{14}N)_{standard}} - 1 \right) 1000 \qquad \text{(Eq. 3.1)}$$

where R_{sample} and $R_{standard}$ refer to the $^{15}N/^{14}N$ ratio measured in the sample and standard of atmospheric N_2, respectively (Chalk et al., 2015). Prior to isotopic analysis, vegetation samples were dried at 40°C and soil samples were separated from bulk root material and air dried. Dried samples were ground using a ball mill and 2-4 mg of vegetation sample and 60-80 mg of soil sample were packed into tin capsules. Gravimetric soil moisture content (θ_d) was determined by drying a 1 g soil subsample at 100°C for 24 h.

3.3.4 Enriched Isotope Experiment

Three blocks on each of the dolomitic and granitic parent material were selected for the enriched isotope study. In each block one diapir and one control frost boil (n=12) were injected with enriched N immediately following snowmelt (between July 12th and 19th). Snowmelt was delayed by ca. 1 to 2 weeks due to low spring temperatures and unusually high winter snowpack and meltwater volumes. A 40 cm^2 injection grid with 72 injection points spaced 3 cm apart was placed at the centre of each frost boil and 1 ml of ^{15}N-labelled ammonium nitrate solution (98 atom% $^{15}N_2$) was injected into the soil at each point on the grid using a syringe attached to a 30 cm perforated needle. The depth of injection corresponded to the depth of high spectra in the control frost boils and to the depth of maximum SOC along the SOC curve in diapir frost boils using the vis-NIR spectra profile. A total of 105 mg of $^{15}NH_4{}^{15}NO_3$ was added to each grid, equalling 238 g N m^{-2}. The amount of $^{15}NH_4{}^{15}NO_3$ added equalled 16.5% of K_2SO_4 extractable NH_4-N and NO_3-N in the soil solution (Brummell et al., 2012). Following an incubation period of three to four weeks, a set of leaf, aboveground stem and belowground stem/root tissue from the three *S. arctica* plants closest to the injection grid (n=9) and within the frost boil boundary were collected between August 6th and 18th. Leaf material was sampled prior to aboveground senescence, approximately one week before stem and root material was collected.

Vegetation samples were prepared for isotopic analysis as described above (Section 3.3.3), however root tissue was rinsed with calcium rich water to remove any enriched label adhered to outer tissue prior to drying. Enriched values were expressed in atom% and defined as:

$$\text{atom\%}^{15}\text{N} = \frac{amount\ of\ ^{15}\text{N}}{amount\ of\ ^{14}\text{N} + ^{15}\text{N}} \qquad \text{(Eq. 3.2).}$$

3.3.5 Data Analyses

Statistical analysis was performed using SAS/STAT® software (Version 9.4, SAS Institute, Cary, NC, USA). Natural abundance and enriched isotope plant samples were analyzed as the average of leaf, stem and root tissues from a single plant. Natural abundance vegetation and soil parameters were analyzed for normality using Shapiro-Wilk's test and non-normally distributed data was transformed using Box-Cox transformations. Mixed effects models were implemented using the MIXED procedure in a randomized complete block design (RCBD). Spectra category, depth increment or vegetation tissue part were tested separately as fixed effects along with frost boil type and their interaction as additional fixed factors and block as a random effect. Residual analysis was performed using the UNIVARIATE procedure with Normal and PLOT options. The restricted maximum likelihood method was used as the variance component estimate and the Kenward-Roger method for approximating degrees of freedom for means. Data with $P < 0.05$ were considered statistically significant but tendencies towards significance $P < 0.10$ were also reported. Fisher's Least Significant Difference (LSD) method was used to compare means when a significant effect was found. To determine the source of nitrogen uptake by *S. arctica* from individual spectra categories within control and diapir frost boils, simple linear regression was performed between soil $\delta^{15}N$ of spectra categories and vegetation $\delta^{15}N$ separately within frost boil types using PROC REG. Slopes of regressions were tested for significance using Student's *t* test.

Enriched isotope atom%^{15}N in vegetation was analyzed as a generalized linear mixed model in an RCBD treatment design using the GLIMMIX procedure and applying a gamma distribution using the LINK function. Vegetation tissue part, frost boil type and their interaction were tested as fixed factors and block as a random effect. The restricted (residual) pseudo-

likelihood method was used as the variance component estimate and the containment method for approximating degrees of freedom for means. LSD method was used to compare means when a significant effect was found.

3.4 Results

3.4.1 Environmental Properties

The surface of frost boils on granitic parent material (Table 3.1) was a mixture of coarse material (i.e. rock and gravel) and fine soil with a thin layer of bryophytes and lichens (predominately crustose with small amounts of foliose and fruticose). *S. arctica* was present in the highest cover on all frost boils with 15 other species occurring at < 1% cover and litterfall was two-fold higher than cover of vascular plants. The degree of cryoturbation activity and sorting varied between frost boils, however ca. 75% occurred as non-sorted circles with the remainder bordered by a rim of sorted rocks (generally in areas of higher slope). Frost boils ranged in diameter from 1.2 to 2.2 m (Table 3.2).

The blocks divided into four groups similar in surface cover and frost boil development: (I) weak development with little to moderate vegetation and a high cover of coarse material and bare soil (i.e. blocks A-E), (II) moderate development with little vegetation and a high cover of bryophytes (i.e. block F and G), (III) moderate development with moderate to high vegetation, decreasing cover of coarse material and increasing accumulation of fine soil (i.e. blocks H-J), and (IV) moderate to high development with high cover of vegetation and accumulation of litter (i.e. block K and L). Total C and total N increased with increasing frost boil development and were highest in wetter areas of high plant and in particular, litter cover (i.e. group IV). High cover of bryophytes (i.e. group II) did not increase total N content compared to frost boils with low bryophyte cover (i.e. group I) that were similar in development.

Table 3.1. Surface characteristics of blocks on granitic parent material. Values are means of surface cover groups and (standard errors).

Group	Block	Rock	Gravel	Bareground	Lichen	Bryophytes	Litter	*S. arctica*	Other Spp.Present‡
					— % cover† —				
I	A-E	13 (2)	9 (1)	20 (2)	14 (1)	35 (2)	12 (2)	6 (1)	LC, FB, PH, LA, SO, CT, SN, CA, DC, PR, DI, PA
II	F-G	8 (2)	7 (1)	11 (2)	9 (1)	58 (3)	10 (2)	5 (1)	LA, PH, SN, FB, CA, JB, LC, DI, DC, PR, CB, SF
III	H-J	7 (1)	7 (1)	18 (3)	15 (1)	41 (3)	16 (2)	9 (1)	CT, LC, LA, FB, PH, SO, DI, SN
IV	K-L	19 (2)	7 (1)	6 (2)	14 (2)	37 (3)	24 (4)	12 (2)	LC, FB, LA, PH, SO, CA, CT, DI
Average		12 (4)	8 (2)	16 (4)	13 (3)	40 (5)	15 (4)	7 (2)	LC, FB, CT, LA, PH, SO, SN, CA, DI, JB, DC, PR, PA, CB, SF

† Classes of surface cover are % cover averages of 25 cm by 25 cm quadrats (n=9) across frost boils (n=2) in each block.

‡ The total of all other spp. present was ≤ 1%. Species are in descending order of % cover. CA = *Cerastium alpinum*, CB = *Cardamine bellidifolia*, CT = *Cassiope tetragona*, DC = *Draba corymbosa*, DI= *Dryas integrifolia*, FB = *Festuca brachyphylla*, JB = *Juncus biglumis*, LA = *Luzula arctica*, LC = *L. confusa*, PA = *Poa arctica*, PH = *Pedicularis hirsuta*, PR = *Papaver radicatum*, SF = *Saxifraga flagellaris*, SN = *Saginella nivalis*, SO = *Saxifraga oppositifolia.*

Table 3.2. Soil characteristics of blocks on granitic parent material. Values of total N, total C and gravimetric soil moisture content (θ_d) are means and (standard errors) of soil cores from four depths in a control and diapir frost boil (*n*=8).

Group	Block	Total N	Total C	θ_d	Frost Boil Diameter	Sorting†	Surface Activity‡
		—g kg^{-1} —		kg kg^{-1}	m		
I	A	0.27 (0.05)	2.7 (0.5)	0.094 (0.009)	1.2	–	++
	B	0.21 (0.04)	2.2 (0.4)	0.087 (0.006)	1.8	–	+
	C	0.20 (0.02)	2.0 (0.3)	0.105 (0.004)	1.9	–	++
	D	0.19 (0.02)	1.9 (0.2)	0.096 (0.009)	1.2	–	–
	E	0.23 (0.01)	2.3 (0.1)	0.085 (0.003)	1.3	+	++
II	F	0.24 (0.03)	2.3 (0.3)	0.094 (0.009)	1.4	+	+
	G	0.22 (0.03)	2.1 (0.3)	0.093 (0.008)	1.4	–	+
III	H	0.27 (0.03)	2.8 (0.4)	0.100 (0.005)	1.6	–	–
	I	0.19 (0.02)	2.0 (0.2)	0.086 (0.010)	2.2	–	+
	J	0.33 (0.04)	3.4 (0.4)	0.097 (0.006)	1.7	–	+
IV	K	0.31 (0.03)	6.5 (1.2)	0.102 (0.007)	1.5	–	+
	L	0.42 (0.14)	4.6 (1.6)	0.102 (0.012)	1.3	+	+
Summary of Surface Cover Groups§							
I	A-E	0.22 (0.02)	2.25 (0.15)	0.093 (0.003)			
II	F-G	0.23 (0.02)	2.19 (0.21)	0.093 (0.006)			
III	H-J	0.26 (0.02)	2.68 (0.21)	0.094 (0.004)			
IV	K-L	0.37 (0.07)	5.50 (1.00)	0.107 (0.008)			

†Refers to the degree of organization in the frost boil, (+) indicating a border of stones, and (-) indicating an undefined border lacking stones.

‡Refers to evidence of recent cryoturbation in the frost boil, with (-) indicating absence of activity and full crust cover, (+) indicating small patches of disturbed cover, and (++) indicating an upheaved center of bare soil.

§Surface cover groups based on similarities in surface cover features according to Table 3.1.

3.4.2 Plant Cover Response to Diapir Presence

Total plant and *S. arctica* cover did not differ between frost boil types ($P > 0.05$) while litter cover had a tendency to increase with diapir presence ($P < 0.10$) (Table 3.3). All cover indicators increased on diapir frost boils, particularly as development progressed. In surface cover groups, (i.e. groups I, III and IV from Table 3.1), the increase in plant and litter on diapir frost boils was proportional to 15 - 51% and 35 - 95% of the total plant and litter cover on control frost boils, respectively (see Appendix A3). The greatest responses occurred within the most well-developed frost boils and decreased in surface cover groups for plant cover in order from IV > III > I and litter cover in order from IV > I > III. Frost boils with high bryophyte cover (i.e. group II) did not show a positive response and decreased in plant and litter cover with diapir presence.

Table 3.3. Total plant, *S. arctica* and litter cover on diapir (*n*=12) and control (*n*=12) frost boils on granitic parent material. Values are means and (standard errors) of each frost boil type.

Type	Total Plant	*S. arctica*	Litter
		— % cover —	
Control	7.2 (0.7)	6.7 (0.8)	11.8 (1.1)
Diapir	9.3 (1.4)	8.2 (1.3)	17.5 (2.7)
Summary Statistics of Frost Boil Type as a Fixed Effect			
df	1, 11	1, 11	1, 11
F-value	2.52	0.83	3.73
P > F	0.141	0.382	0.080

† Classes of surface cover are % cover averages of 25 cm by 25 cm quadrats (n=9) on each frost boil.
‡ Degrees of freedom (numerator df, denominator df).

3.4.3 Soil Nutrient Content

Soil nutrient content was low within frost boils, averaging 2.9 ± 0.2 g C kg^{-1} dry soil (mean ± SE), and 0.26 ± 0.02 g N kg^{-1} dry soil. Total C ($P < 0.05$) and total N ($P < 0.10$) varied with depth and changed correspondingly through the soil profile. Total C and N were present in greatest amount at the surface and declined to 15 – 25 cm depth before increasing in content above the permafrost table (Table 3.4). C:N ratios were similar through the soil profile ($P > 0.05$) and decreased slightly with depth. Soil moisture was lowest from 0 – 15 cm depth and had a tendency to increase towards the permafrost table ($P < 0.10$).

The vis-NIR spectra readings overestimated SOC in the field and were over two-fold higher than total C, averaging 6.9 ± 0.4 g OC kg^{-1} dry soil. The overestimation was likely influenced by moisture content (Guy et al., 2015). Moisture did not vary significantly through spectra categories ($P > 0.05$), thus the spectra readings were able to consistently predict the relative OC amount in the field (Table 3.5). Field SOC decreased from high SOC (i.e. surface and high spectra categories) to low SOC (i.e. medium and low spectra categories) sources ($P < 0.001$) (Table 3.5).

Total C and total N were greater in diapir frost boils, although nutrient content did not differ significantly between frost boil types ($P > 0.05$) (Table 3.5). The distribution of total C ($P < 0.05$) and total N ($P < 0.10$) decreased from high to low SOC sources in order from surface > high > low > medium within spectra categories on all frost boils (Figure 3.2B). Total C and N were highest at the diapir surface followed by the diapir Bhy horizon (Table 3.5). The high SOC sources in controls had lower total C and total N relative to respective diapir categories. Low SOC sources were similarly depleted in total C and total N on control and diapir frost boils. Soil moisture was highest in the low spectra category in both frost boil types.

Table 3.4. Soil characteristics of frost boils on granitic parent material according to depth. Values are means and (standard errors) of soil cores from depth categories (*n*=22-27) within all frost boils.

Depth	Total C	Total N	C:N	$\delta^{15}N$	θ_d
	—g kg^{-1} —			‰	kg kg^{-1}
0 – 5	3.8 (0.6) a	0.32 (0.04)	10.2 (0.1)	5.4 (0.2) a	0.092 (0.005)
5 – 15	2.6 (0.3) b	0.23 (0.02)	10.3 (0.1)	5.8 (0.2) b	0.088 (0.004)
15 – 25	2.4 (0.2) b	0.22 (0.01)	10.1 (0.1)	6.1 (0.1) bc	0.095 (0.004)
25 – 35	2.9 (0.5) b	0.26 (0.04)	9.9 (0.1)	6.3 (0.2) c	0.104 (0.004)
Summary Statistics of Depth as a Fixed Effect					
df	3, 82	3, 82	3, 73	3, 80	3, 91
F-value	2.72	2.28	1.99	10.4	2.28
P > F	0.049	0.086	0.120	< 0.001	0.085

Letters represent differences between means using the LSD method ($P < 0.05$).

3.4.4 Soil $\delta^{15}N$ Profiles

Soil $\delta^{15}N$ had a narrow range from 5 to 7‰ and increased with depth ($P < 0.001$) (Table 3.4). Total N was negatively correlated with $\delta^{15}N$ signatures in spectra categories and increased in order from surface < high < medium < low ($P < 0.001$) (Figure 3.3B). $\delta^{15}N$ signatures in spectra categories were similar between frost boil types ($P > 0.05$) (Table 3.6), however the distribution range in spectra categories varied. In diapir frost boils, the high SOC sources were depleted and low SOC sources enriched in $\delta^{15}N$ signatures relative to controls (Figure 3.4B).

3.4.5 Plant N

The amount of N in *S. arctica* plants did not differ between control and diapir frost boils ($P > 0.05$). Total N was highest in leaf (25 ± 0.3 mg N g^{-1} dry tissue), decreasing four-fold in the aboveground stem and belowground stem/roots (6 ± 0.1 mg N g^{-1} dry tissue) (Figure 3.3A).

Table 3.5. Soil characteristics of frost boils on granitic parent material according to spectra category. Values are means and (standard errors) of soil cores from spectra categories (*n*=12) within control and diapir frost boils.

Spectra Category	Field SOC†	Total C	Total N	θ_d
		—g kg^{-1} —		$kg\ kg^{-1}$
Control				
Surface‡	7.1 (1.0)	3.1 (0.5)	0.27 (0.03)	0.090 (0.008)
High‡	9.0 (1.7)	3.0 (0.7)	0.25 (0.03)	0.095 (0.005)
Medium§	7.4 (1.1)	2.5 (0.4)	0.22 (0.02)	0.093 (0.006)
Low§	4.9 (0.6)	2.6 (0.4)	0.23 (0.01)	0.104 (0.007)
Diapir				
Surface‡	7.2 (1.2)	4.5 (1.1)	0.37 (0.08)	0.093 (0.006)
High‡	8.2 (1.3)	3.2 (0.8)	0.30 (0.07)	0.089 (0.007)
Medium§	6.2 (0.6)	2.1 (0.2)	0.20 (0.02)	0.096 (0.006)
Low§	5.0 (0.5)	2.3 (0.3)	0.23 (0.02)	0.098 (0.005)
Summary of Frost Boil Type				
Control	7.1 (0.6)	2.5 (0.3)	0.24 (0.01)	0.096 (0.003)
Diapir	6.6 (0.5)	3.0 (0.4)	0.28 (0.03)	0.094 (0.003)
Summary Statistics of Fixed Effects				
Frost boil				
df	1, 73	1, 77	1, 77	1, 87
F-value	0.15	0.05	0.03	0.10
P > F	0.700	0.821	0.859	0.753
Spectra				
df	3, 73	3, 77	3, 77	3, 87
F-value	18.81	3.26	2.64	1.05
P > F	<0.001	0.026	0.055	0.376
Frost boil x spectra				
df	3, 73	3, 77	3, 77	3, 87
F-value	0.95	0.74	0.67	0.39
P > F	0.421	0.523	0.570	0.762

† Predicted amount of SOC measured in situ by vis-NIR spectra at Dome site.

‡ Surface and high spectra categories represent high SOC sources based on vis-NIR spectra profiles. Surface spectra is sampled from 0 to 5 cm depth and represents inputs primarily from litterfall and N_2 fixation by organisms in the soil crust. High spectra represents a high SOC source that does not occur on a SOC peak in a control frost boil or a peak of the SOC curve of a diapir horizon in a diapir frost boil.

§ Medium and low spectra categories represent low SOC sources based on vis-NIR spectra profiles.

Table 3.6. Summary statistics of fixed effects for soil δ15N, plant δ^{15}N, and plant atom% ^{15}N.

Factor	df	F-value	*P* > F
Soil $\delta^{15}N$			
Frost boil	1, 75	0.03	0.871
Spectra	3, 75	9.01	< 0.001
Frost boil x spectra	3, 75	0.59	0.625
Plant $\delta^{15}N$			
Frost boil	1, 54	0.7	0.408
Tissue part	2, 54	12.3	<0.001
Frost boil x tissue part	2, 54	0.01	0.985
Plant atom%^{15}N			
Frost boil	1, 30	13.35	0.001
Tissue part	2, 30	0.82	0.448
Frost boil x tissue part	2, 30	0.50	0.610

3.4.6 Plant δ^{15}N Profiles

The δ^{15}N signatures of *S. arctica* tissue were ^{15}N-depleted and ranged from -3 to 0‰ (Figure 3.3A). Vegetation δ^{15}N signatures were negatively correlated with total N content that decreased in order from leaf > stem > root. There was significant intra-plant variation between δ^{15}N signatures in tissue parts ($P < 0.001$) (Table 3.6) and the greatest change in δ^{15}N signatures occurred between belowground stem/root and aboveground stem tissues that had equal N content (Figure 3.3A). Tissue δ^{15}N signatures were highest in the root and decreased considerably in aboveground stem ($\Delta_{root-stem}$ = 1.5‰) and leaf ($\Delta_{root-leaf}$ = 1.7‰). Intra-plant variation was greater in diapir ($\Delta_{root-leaf}$ = 1.8‰) than control ($\Delta_{root-leaf}$ = 1.6‰) frost boils (Figure 3.4A) even though plant N content did not differ between frost boil types ($P > 0.05$).

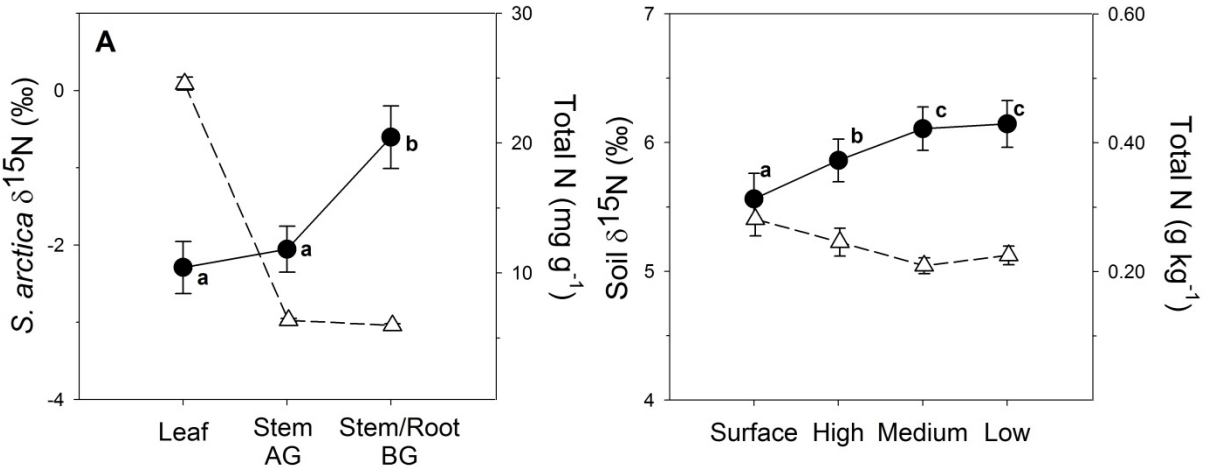

Figure 3.3. Natural abundance $\delta^{15}N$ signatures ± SE (circles) and total N ± SE (triangles) of *S. arctica* leaf, aboveground stem and belowground stem/root tissue (Panel A) and soil spectra categories (Panel B) on all frost boils. Letters represent differences between means and were calculated using the LSD method ($P < 0.05$).

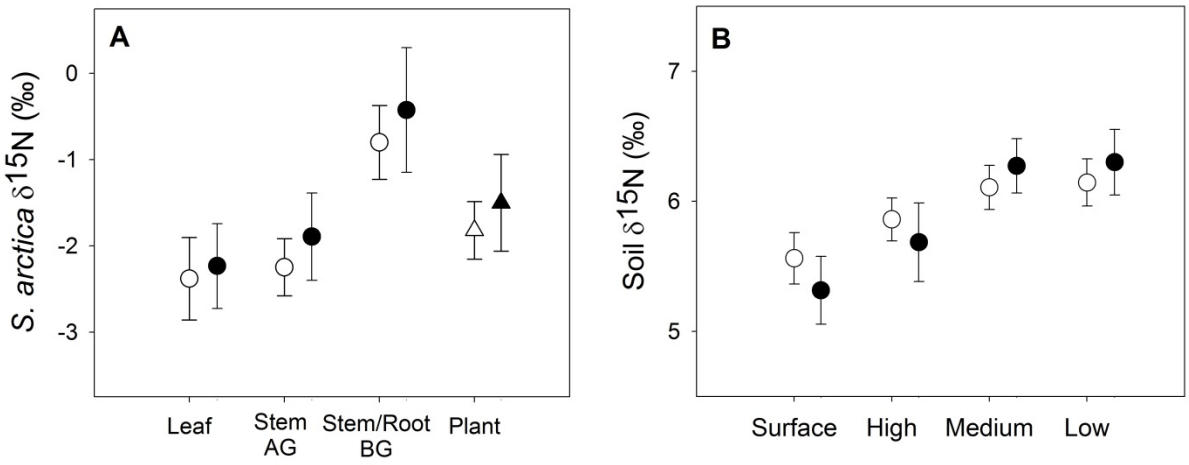

Figure 3.4. Natural abundance $\delta^{15}N$ signatures ± SE in control (open symbol) and diapir (closed symbol) frost boils of *S. arctica* (Panel A) leaf, aboveground stem and belowground stem/root tissue (circles) and average plant (triangles) and soil spectra categories (Panel B).

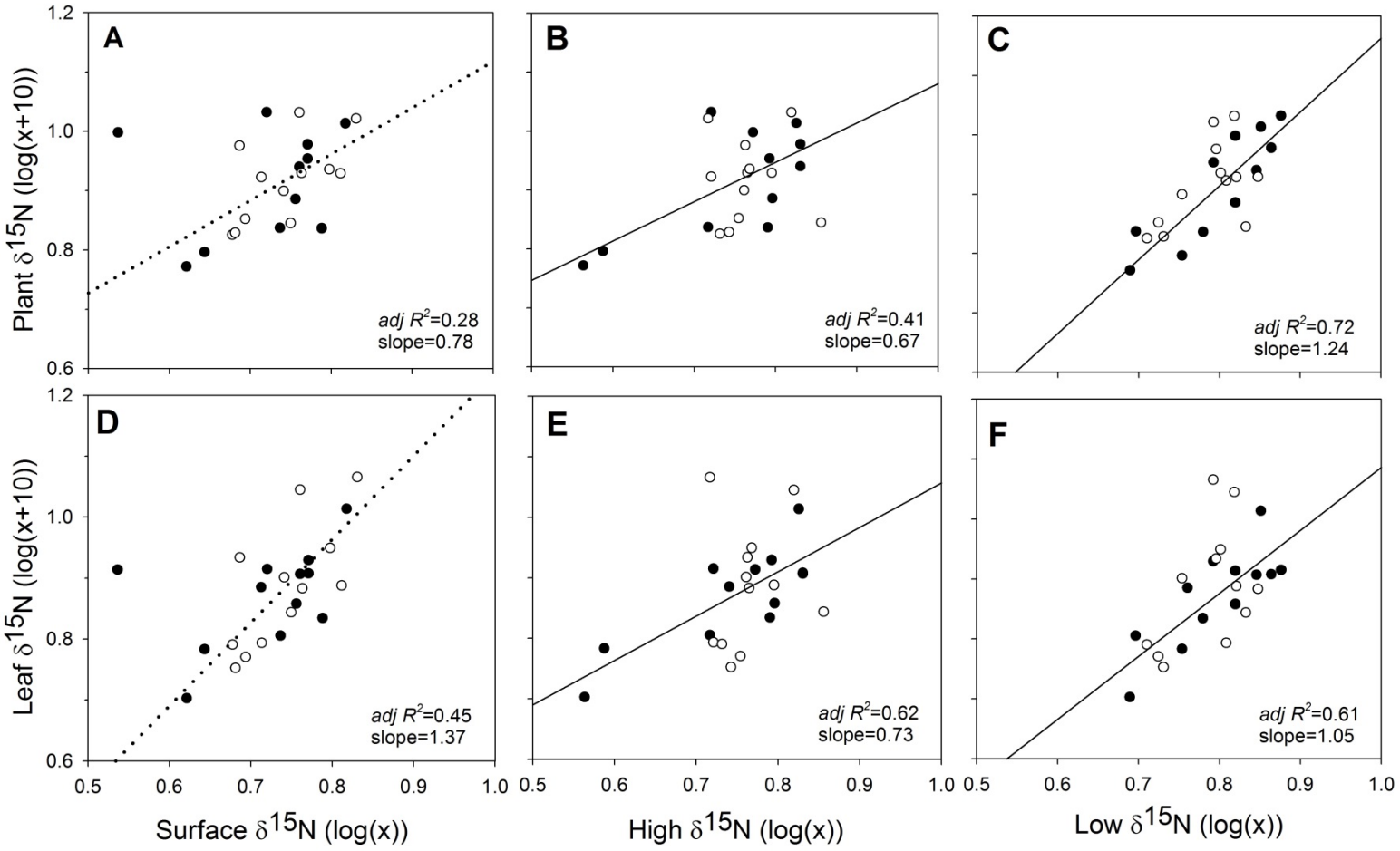

Figure 3.5. Nitrogen uptake was determined by plotting the regression of natural abundance $\delta^{15}N$ *S. arctica* signatures against soil source spectra signatures in control (open circles; dotted regression lines) and diapir (closed circles, solid regression lines) frost boils. Adjusted R^2 and slope values are reported for significant regressions ($P < 0.05$) and non-significant regressions are indicated by the absence of a line.

3.4.7 N uptake by *S. arctica* in Frost Boils

S. arctica signatures – though similar between frost boil types ($P > 0.05$) – were consistently higher on diapir frost boils (Figure 3.4A) and may indicate that plants are utilizing different nutrient acquisition strategies depending on the type of frost boil they are located on. Low $\delta^{15}N$ signatures in plants located on control frost boils may be caused by increasing uptake of depleted $\delta^{15}N$ at the surface. Alternatively, the enriched $\delta^{15}N$ signatures in diapir vegetation may suggest plants are reducing their reliance on surface N and utilizing a mixture of soil sources that are also $\delta^{15}N$-enriched. This relationship is indicated in Figure 3.5, where $\delta^{15}N$ of *S. arctica* plants located on control frost boils were positively correlated with $\delta^{15}N$ at the soil surface (Figure 3.5A and D) with no association to N sources deeper in the soil. In diapir frost boils, $\delta^{15}N$ of *S. arctica* plants was positively correlated with the diapir horizon (Figure 3.5B and E), and low N sources (Figure 3.5C and F), with no association to the soil surface.

The ability of *S. arctica* to take up N directly from the diapir horizon was clearly demonstrated with enriched isotope sites (Figure 3.6). *S. arctica* was able to accumulate substantially more N over the growing season from diapir horizons than the high N soil source of controls and average plant atom%^{15}N was 2.5 fold higher in diapir frost boils ($P < 0.01$) (Table 3.6). Tissue atom%^{15}N decreased in order from leaf > root > stem in plants on diapir frost boils and leaf > stem > root on control frost boils. *S. arctica* transferred over 70% of the acquired ^{15}N to aboveground tissue in both frost boil types and accumulation was highest in leaf tissue of diapir plants (ca. 50% of total plant uptake).

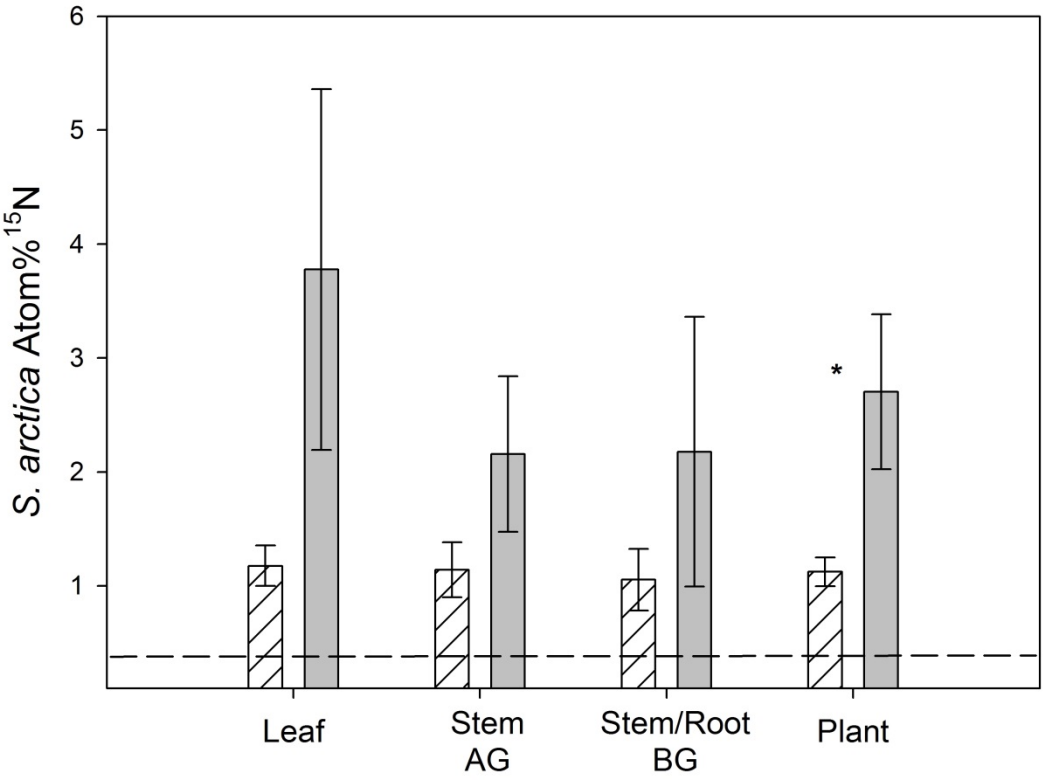

Figure 3.6. Enriched isotope atom%^{15}N signatures ± SE of *S. arctica* tissue on control (white diagonal stripe) and diapir (solid gray) frost boils. The black dashed line indicates the natural abundance level of plant uptake (0.3663 atom%^{15}N). There was a significant difference between average plant tissue means ($P < 0.05$; indicated by *) of control and diapir frost boils.

3.5 Discussion

3.5.1 Distribution and Availability of N in Diapirs

In frost boils – as in the majority of natural soil environments – available nutrients are distributed in heterogeneous patches that fluctuate spatially and through time (Hodge, 2006). Although average N was similar across frost boils, the size, location and concentration of N-rich patches differed. The diapir (Bhy) horizon often forms below the center of the frost boil as a spatially-variable nutrient patch resembling a plume several decimetres in length (Swanson et al., 1999; Ugolini et al., 2006). Based on vis-NIR spectra readings at this site, the Bhy typically ranged from 5 to 15 cm in length with the peak of SOC often within 20 cm of the surface.

Compared to other subsurface high N sources (usually ≤ 5 cm in length), diapir horizons were relatively large patches of higher contrast to background fertility and after the soil surface, provided the second largest source of N in this polar desert (Table 3.5).

The intermediate $\delta^{15}N$ signature of the diapir horizon (Figure 3.4B) indicates the Bhy is a mixture of N originating from both the surface and permafrost layers and varies according to the relative proportion of inputs from each source and degree of biological processing (Robinson, 2001). At the soil surface, N content is highest (Figure 3.3B) and derives largely from decomposition of litter (mainly from *S. arctica*, with small contributions from other species), and N_2-fixation by bryophyte-cyanobacterial associations in the soil crust (Stewart et al., 2011a) (Table 3.1 and 3.2). Following snowmelt, dissolved N from the surface can leach with meltwater through the soil profile (Tye et al., 2005; Schaeffer et al., 2013) and accumulate in the concave-shaped bowl occurring above the center of the permafrost table (Cannone et al., 2004; Walker et al., 2004; Boike et al., 2008). The accumulation of water causes the uppermost permafrost to thaw and form a slurry that varies in the relative proportion of N from the surface and permafrost and is reflected in the wide $\delta^{15}N$ range of the diapir Bhy (Figure 3.4B). Plant litter and biologically fixed N_2 are ^{15}N-depleted and the $\delta^{15}N$ signature of surface N is often the lowest in the soil profile (Högberg et al., 1996; Rhoades et al., 2008; Stewart et al., 2011b). The permafrost layer was not characterized at this site, however SOC and N have been found in similar amounts throughout the active and permafrost layers in other polar desert environments (Michaelson et al., 2008; Ping et al., 2008a; Steven et al., 2008; Palmtag et al., 2015). Although SOC in permafrost is largely recalcitrant, a portion of the pool is relatively labile (Ping et al., 2015). Permafrost harbours a diverse microbial population that is able to mineralize N at a low level of activity (Panikov et al., 2006; Steven et al., 2008; Yergeau et al., 2010; Keuper et al.,

2012). Prior to thaw and redistribution in the slurry, the $\delta^{15}N$ of permafrost derived-N is likely to be lower than stable N sources in the active layer that have undergone substantial microbial processing (i.e. low SOC sources). As decomposition of SOM and mineralization of N proceeds with time, $\delta^{15}N$ signatures increase and eventually begin to reflect the microbial alteration of organic N compounds and turnover of the ^{15}N-enriched microbial biomass (including mycorrhizal hyphae) (Kramer et al., 2003; Hobbie and Hobbie, 2006; Dijkstra et al., 2008; Wallander et al., 2009; Lerch et al., 2011). Active populations of microorganisms have been reported deep in the active layer at this site (Brummell et al., 2012); as the diapir Bhy develops upon movement into the active layer, $\delta^{15}N$ increases with SOM mineralization in the warming soil (Biasi et al., 2005; Nowinski et al., 2010; Mackelprang et al., 2011; Mueller et al., 2015).

The formation of diapir horizons depend upon many different factors that are also related to its movement and availability in the soil. As water accumulates above the impermeable permafrost layer, unstable bulk density profiles lead to the upward flow of material at the permafrost table (Swanson et al., 1999; Bockheim, 2007). In northern Alaska, active layer soil with a moisture content 10-fold higher than that observed at Dome site during mid-growing season is conducive to diapir formation (Swanson et al., 1999). Although moisture is limiting through polar deserts, water accumulates in frost boils above a shallow permafrost table that is typically within 0.5 m of the surface (Bliss, 1994; Ugolini et al., 2006; Brummell et al., 2012). The accumulation of water is particularly high following a winter of high snowfall as meltwater slowly drains laterally through the landscape over the growing season. In a particularly warm, wet and active year – a diapiric injection could form and potentially shift out of the permafrost or higher in the frost boil at an average rate of 0.01 m day^{-1} (Swanson et al., 1999). A diapir horizon that may have been unavailable to plants one year could become available the next as it is

vertically heaved towards the surface over the subsequent freeze/thaw cycles (Overduin and Kane, 2006; Walker et al., 2008). Although the time necessary for diapir formation is unknown, the frequency of formation events are likely to increase as the arctic environment changes (Callaghan et al., 2005; Bockheim, 2007) and multiple diapiric injections may become superimposed within the soil profile to increase N availability in frost boils through time (Phillips, 2011).

3.5.2 Plant Response to Patchy Diapir Nutrients

Plants are able to respond to the spatial and temporal variations in nutrient availability by altering root growth patterns (McNickle et al., 2009; Cahill and McNickle, 2011). When encountering a nutrient-rich area many plant species are able to rapidly deploy fine roots (< 2.0 mm in diameter) to precisely exploit the patch (Hodge, 2004; Sullivan et al., 2007), particularly if the patch is large or of high contrast to background fertility (Hutchings et al., 2003). In the absence of diapir horizons, roots were unresponsive to small N-rich patches deeper in the soil and *S. arctica* relied on N patches at the surface arising from litter decay and biologically fixed-N_2 (Figure 3.5). Although root elongation is slow in polar desert plants, averaging 1.0 to 1.2 cm yr^{-1} (Bell and Bliss, 1978), many roots extend to 15 cm depth up to a maximum of 25 cm (Iversen et al., 2015), well within the diapir horizon range (i.e. 10 to 35 cm depth). Active rooting in this zone is confirmed by the uptake of enriched-atom%^{15}N nutrients (Figure 3.6). As diapir horizons became available, the majority of uptake no longer occurred from the surface but rather from the diapir horizon (Figure 3.5). The 2.5 fold increase in atom%^{15}N uptake from diapir horizons suggest *S. arctica* either selectively concentrated roots in the N-rich patch or actively increased the nutrient uptake rates of existing roots within the diapir (Jackson et al.,

1990) (Figure 3.6) further supported by soil cores from Dome site showing increasing root biomass present in the diapir horizon (Guy, pers. comm.).

The quality of SOM inputs and timing of N release relative to the plant's demand may account for the preferential utilization of diapir nutrients by *S. arctica* when considerable N appeared to be available at the surface. Inputs from N_2-fixation by biological soil crusts are controlled by environmental conditions such as moisture and temperature that vary through the growing season (Zielke et al., 2005; Lett and Michelsen, 2014). Although bryophyte-cyanobacterial associations are able to fix N_2 at rates 1.5 to 2-fold higher at Dome site than other low-latitude arctic areas (Stewart et al., 2011a), considerable fixed N_2 usually only accumulates within a few days of snowmelt during late June to early July (Dickson, 2000), and a high proportion may be lost through both leaching (Tye et al., 2005; Yano et al., 2010; Schaeffer et al., 2013; Steven et al., 2013) and gaseous release during volatilization and denitrification (Ma et al., 2007; Brummell et al., 2014). These early season N pulses at the surface from increased N_2-fixation rates along with microbial dieback during snowmelt (Schmidt and Lipson, 2004; Buckeridge and Grogan, 2010; Larsen et al., 2012) and translocation of stored N from belowground storage compartments (Maessen et al., 1983; Andresen and Michelsen, 2005) are important for the rapid production of leaves in deciduous species at spring (Tolvanen and Henry, 2001). The period of leaf production is followed by woody stem production (Shaver, 1986) and maturation over the remainder of the growing season (Ardnal et al., 2009). Nutrient inputs from litter at the surface are also ephemeral with a pulse of free amino acids and easily-mineralizable N released from senesced leaves early in spring (Schmidt and Lipson, 2004) and late in the season when plant demand is low (Kielland, 1994; 1995). Slow decomposition of low quality litter, particularly woody material with high lignin, polyphenols (e.g. tannins) and C:N ratios

typical of inputs under deciduous heath, can result in the release of low levels of plant-available nutrients by the microbial community throughout the growing season (Hobbie, 1996; Hobbie and Gough, 2004; Weintraub and Schimel, 2005; Eskelin et al., 2009; Osono et al., 2014). Due to these seasonal limitations in N availability, *S. arctica* likely relies on subsurface N patches to satisfy mid-season N demands, which peak between early to late July in the high arctic (Tolvanen and Henry, 2001; Ardnal et al., 2009). The accumulation in the present study of ca. 50% of acquired enriched-^{15}N originating from the diapir horizon in leaves prior to senescence in mid-August supports this pattern of N demand (Figure 3.6). The atom%^{15}N enriched nutrients were applied following snowmelt (which was delayed due to an enhanced winter snowpack and low spring temperatures) in mid-July after the initial expansion of leaves had occurred. Any subsequent N accumulations in leaves would likely be used to support photosynthesis (Baddeley et al., 1994) or production of new biomass through apical or secondary stem growth (Campiolo et al., 2012). If supply temporarily exceeded demand, some N may have been stored for later translocation into belowground tissue (Maessen et al., 1983). *Salix* spp. are very responsive to the increasing availability of N within polar deserts and increase surface cover (Arens et al., 2008) and leaf density (Madan et al., 2007) in response to low levels of fertilization. Physiological changes including increased N leaf concentration and photosynthetic rate have also been observed (Baddeley et al., 1994; Jones et al., 1997). The increased uptake of diapir-derived N likely facilitated the production of aboveground tissue and subsequent increase in plant cover and litter deposition at this site (Table 3.3). Furthermore, these responses may negatively affect the SOM quality at the surface and further enhance the dependence on the diapir horizon to supply N. The accumulation of low quality litter inputs are indicated at the diapir frost boil surface by depleted δ^{15}N values (Figure 3.4B) and high total C, N and C:N ratios (Table 3.6) that

are also likely low in soluble N:phenolics ratios (Eskelin et al., 2009). Conversely, the lack of additional litter inputs indicated by the enrichment in soil $\delta^{15}N$ and stabilization of total C and N budgets in the absence of diapir horizons (Table 3.6), permitted the continual uptake of N from the surface (Figure 3.5A and D).

The association of plant $\delta^{15}N$ with low SOC sources in the diapir frost boil indicate plants are still placing roots outside of N-rich patches at various depths (Figure 3.5). Increased N uptake from roots in N-depleted zones could follow the pulses of mobile N such as dissolved organic nitrogen (DON) and NO_3^- that are transported deeper in the soil during periods of increased hydraulic conductivity (Tye et al., 2005; Schaeffer et al., 2013). In polar deserts, the early season flush of N from microbial dieback (Schmidt and Lipson, 2004; Buckeridge and Grogan, 2010; Larsen et al., 2012) and N_2-fixation (Dickson, 2000) in meltwater rapidly flow to underlying roots (up to 8-fold faster than diffusion alone) (Chapin et al., 1988). Roots can respond to these nutrient pulses by temporarily adjusting their inflow rate and increasing uptake capacity (Jackson, 1990; Caldwell et al., 1992; Ivans, 2003; James et al., 2009), though pre-pulse root system size is a key determinant of the benefit a plant can obtain from an ephemeral nitrogen pulse (Lamb et al., 2012). Additionally, low SOC sources contained the highest proportion of moisture within frost boils (Table 3.3) and may have influenced the rooting of *S. arctica* into these regions. The foraging response of plants to moisture patches is not as well understood as with soil nutrients (Cahill and McNickle. 2009), yet if plants were to react similarly to spatial variability in water resources this would suggest *S. arctica* may be actively foraging for both N-rich and water-rich zones. However, if the continual uptake of N by water foraging and physiological adjustments in response to temporally-variable N pulses was facilitated in *S. arctica* by the increasing utilization of low SOC sources, the use of similar

foraging strategies would be expected to occur in plants across all frost boils. The lack of a strong association between control plants and the low spectra source (Figure 3.5 C and F) suggests the presence of the diapir Bhy may cause an increase in the utilization of this low N source. Low spectra sources were often located between the diapir Bhy and permafrost and upon locating and proliferating into the diapir Bhy in response to nutritious cues, some *S. arctica* roots may have continued growing deeper into the soil. The subsequent exploration of roots into low N sources may have been influenced by the moisture gradient that increased down the soil profile (particularly as moisture limitations increase later in the season) and/or the accumulation of N forms originating from the overlying Bhy and surface horizons (transported downwards by leaching) or from the underlying permafrost table during small diapiric or cryogenic events (Boike et al., 2008). However, without fully characterizing the fine-scale rooting distributions and isotopic signatures (e.g. $\delta^{15}N\text{-}NO_3$, $\delta^{15}N$-DON) and relative proportion of N forms used by plants throughout these sources, the combination of factors that cause increasing utilization of low SOC sources remains unclear.

3.5.3 Effects of Climate on Frost Boil Ecosystems in Polar Deserts

Conditions favorable for increased cryogenic activity and frost boil development are expected to increase across the arctic by the end of the century (Callaghan et al., 2005). As temperatures are predicted to increase by up to 8°C, the active layer will thicken and substantial permafrost degradation will occur (IPCC, 2013). Increasing winter snowfall in the high arctic and higher volumes of meltwater over the growing season will provide a greater opportunity for the accumulation of ice-rich water at the permafrost table and subsequent diapir formation in polar deserts as deeper summer thaw causes the high-density overburden to slump. If surface moisture is increased for longer periods over the growing season from heavy snowfall, N_2-

fixation by the soil crust may also proportionately increase (Stewart et al., 2011a) and provide more available N in ice-rich water at the permafrost table (Schaeffer et al., 2013). With a higher prevalence of diapir horizons and increasing amounts of available SOC from a thickening active layer, nutrient availability may also increase within frost boils, particularly if higher temperatures drive greater mineralization rates. There is significant uncertainty in the soil responses, however, as the responses of other soil communities at Alexandra Fiord to long-term (> 15 years) manipulations of temperature and fertilization have been very slow (Lamb et al., 2011). Arctic plant communities, particularly those dominated by deciduous shrubs such as *S. arctica*, are able to respond more rapidly to environmental changes than soils (Walker et al., 2006; Hudson et al., 2011; Lamb et al., 2011). In polar deserts, plants respond to increasing temperature and nutrient availability by increasing the size, density, photosynthetic rate and N concentration of foliage (Baddeley et al., 1994; Madan et al., 2007; Arens et al., 2008) along with sexual reproductive effort (Klady et al., 2011). The degree of plant response is species specific and more pronounced in shrubs and graminoids relative to forbs. Plant cover was positively affected by the presence of diapir horizons (Table 3.3) in the majority of surface cover groups at this site (i.e. groups I, III and IV). The diapir effect was most pronounced in well-developed frost boils, increasing plant cover by 4 to 5% (equal to a ca. 50% increase in total plant cover on the frost boil) in moderate to highly developed boils. Although ca. 60% of frost boils surveyed are currently weakly developed (i.e. groups I and II), the majority of these frost boils show evidence of recent activity (Table 3.2). Litter inputs proportionately increase with increasing plant cover and a litter feedback loop is expected to increase the carbon balance and further enrich total N in the soil (Walker et al., 2006; Buckeridge et al., 2010). Increased low quality litter inputs with high C:N and low soluble N:phenolics ratios that accumulate from *S. arctica* may reduce overall SOM

quality at the surface and increase the dependency on N derived from the diapir horizon. The findings at this site and across the arctic suggest future climatic conditions that promote diapir formation and frost boil development in polar deserts are likely to also promote increased plant cover in these relatively barren ecosystems.

4. SUMMARY AND CONCLUSIONS

4.1 Summary of Findings

At a polar semi-desert site in the Canadian High Arctic, the occurrence of diapiric intrusions formed from the re-distribution of SOC originating at the permafrost table, affected the N acquisition strategies of the dominant plant species within the frost boil community, the deciduous shrub *S. arctica*. Total C and N were heterogeneously distributed in the soil environment within frost boils and the soil surface contained the highest proportion of N from litter accumulation and N_2-fixation by biological soil crusts. Below the soil surface, the diapir horizon represented the largest N-enriched patch available to plant roots, often occurring mid-profile within 20 cm of the surface and permafrost table. *S. arctica* was responsive to increasing nutrient availability and utilized different N sources depending on the type of frost boil it was located on. Diapir nutrients were a preferred source of N for uptake in *S. arctica* and plants took up considerable N – a 2.5 fold increase over controls – and appeared to increase foraging precision and selectively place roots into these large patches that were of greater contrast to sub-surface background fertility. Interestingly, considerable uptake also occurred from relatively N-limited sources within diapir frost boils and roots may be proliferating into these areas in search of water and/or labile N that has been transported from overlying horizons or underlying permafrost. In the absence of diapirs, plants relied on N available at the soil surface and uptake and/or root selectivity was not cued by any particular N source deeper in the soil.

Frost boils occurred primarily as non-sorted circles that varied in surface cover, activity and development across the landscape. Total C and N increased with increasing frost boil development, vegetation cover and litter accumulation. The presence of diapir nutrients had a positive effect on plant and litter cover with the largest increases occurring in the most well developed areas. Natural abundance $\delta^{15}N$ signatures in the soil revealed that the diapir nutrient zone was a mixture of sources and reflected the relative proportion of N from the relatively ^{15}N-depleted surface and ^{15}N-enriched recalcitrant material originating from the permafrost table. The combination of different labile and recalcitrant inputs from these two sources likely act to sustain N availability in the horizon over a greater portion of the growing season relative to ephemeral N release at the surface and account for the preferential utilization of diapir nutrients by *S. arctica* when larger amounts of N occur shallower in the profile.

The arctic region is changing and although some changes are expected to occur widespread throughout the region (i.e. increasing daily and seasonal temperatures), regional variations in precipitation and permafrost extent are likely to be non-uniform (IPCC, 2013). In high latitude regions dominated by polar deserts, warming air temperatures and increasing winter snowfall are expected to substantially degrade permafrost by the end of the century. These conditions are conducive to the formation of diapir horizons as meltwater over the growing season will add to the accumulation of ice-rich water with depth along with increasing active layer thaw that will degrade the permafrost table and lead to unstable bulk density profiles. Arctic plants have shown rapid aboveground responses to warming and fertilization in these regions at time scales $\leq$ 15 years, particularly in deciduous shrub communities (Walker et al., 2006; Madan et al., 2007; Hudson et al., 2011; Klady et al., 2011; Lamb et al., 2011) and an increase in diapir prevalence across the landscape may positively affect plant growth of certain

species, although the long-term response remains uncertain as the residency time of the diapir patch as a source of plant-available nutrients is unknown. Cryogenic activity that leads to greater frost boil development is also predicted to increase (Callaghan et al., 2005; Bockheim, 2007) and provide a more suitable soil environment (i.e. greater sorting and development of fine-texture particles in frost boils) to expand the growth of plants in polar desert ecosystems.

4.2 Future Research Directions

While this is the first investigation to directly observe the potential of diapir horizons to act as an ecologically important N source for N-limited polar desert plants, further investigations are needed to expand the knowledge of whether it is similarly enriched in P as well as its bio-chemical characteristics and sustainability as a plant nutrient source. Characterizing the presence and amount of specific labile (e.g. NH_4^+, NO_3^-, PO_4^{2-} free amino acids) and recalcitrant (e.g. aliphatic lipids, lignin) N and P forms through time would allow a better understanding of the seasonal and long-term dynamics of nutrient release and residency time of the diapir horizon. Determining whether or not a significant microbial-plant feedback loop exists and quantifying the relative enrichment by different inputs (e.g. turnover of mycorrhizal hyphae versus fine roots) is crucial to determining the sustainability of plant-available nutrient release and presents an interesting area of further study (Luster et al., 2009).

As it is now clear that *S. arctica* plants are able to access diapir nutrients at this polar desert site, it would be of particular ecological interest to determine what other plant species also commonly utilize these nutrients. If a greater number of species that typically grow in low cover (such as forbs and graminoids) were able to simultaneously access diapirs, species diversity may increase within frost boil communities with diapir presence. However, if accessibility was limited to plants such as *S. arctica*, or other dominant species common across the arctic such as

Betula nana, *C. tetragona* and other *Salix* spp., species diversity would likely decrease with the expansion of these large branching shrubs (Walker et al., 2006; Post et al., 2009; Myers-Smith et al., 2011). Understanding which species can respond to diapir horizons will be useful in predicting the trajectory of arctic plant communities as these polar desert environments change.

This is one of relatively few studies to directly observe diapir horizons in the arctic and a large knowledge gap exists in understanding the prevalence of these formations as their occurrence is unknown throughout the region. Frost boil ecosystems and other forms of patterned ground (i.e. ice wedge polygons, earth hummocks, frost mounds) are diverse soil environments that vary considerably throughout the arctic from unvegetated to completely vegetated circles, polygons or stripes (Bockheim, 2015). Whether diapirs are typically common only in polar desert frost boils (Ugolini et al., 2006) or whether they form in other types of patterned ground throughout the arctic is a further area of study that needs to be addressed. Additionally, the variations in characteristic forming factors such as permafrost depth, thaw, moisture and cryoturbation activity, and the typical period and frequency of conditions necessary for diapir formation (Swanson et al., 1999) are not fully characterized across the arctic, even in these dry polar regions. If diapirs are able to form in other forms of patterned ground throughout different arctic ecosystems, the diversity of these environments would lead to the assumption that diapir horizons too would be diverse in characteristics from the different nature of inputs, biological processing rates, degree of cryogenic activity and many other soil properties. If diapirs form in less N-limiting systems with greater SOM inputs, do they play an ecologically important role in plant uptake? Characterizing the formation factors and prevalence of diapir horizons throughout this region is an important area of investigation that is vital to understanding soil-plant interactions as climatic effects become more widespread throughout this changing arctic.

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APPENDICES

APPENDIX 1

A1.1 Summary of $^{18}O_P$ Methodology

P is often co-limiting with N on the growth and establishment of plants within the soil environment of the arctic (Weintraub, 2011) and greater P availability can increase the response of plants to enhanced N within polar deserts (Madan et al., 2007). Diapir horizons are a potential plant-available source of P and a further objective of this project was to characterize P_i (i.e. PO_4^{3-}) uptake from the diapir Bhy using the isotopic signature of $^{18}O_p$. The majority of P in the soil is bound to oxygen (primarily as orthophosphate (PO_4^{3-} or P_i)) and oxygen has three stable isotopes (^{16}O, ^{17}O and ^{18}O). The $^{18}O/^{16}O$ isotope ratio of O bound to P_i (referred to as $^{18}O_P$) is becoming increasingly more common as a tracer of P in the environment, although limited investigations have been made in the soil-plant system (Tamburini et al., 2014). Soil and plant P_i was extracted and purified from samples that were collected at Dome site using the sampling scheme outlined in Section 3.3.3. However, after adapting multiple methods to these polar desert samples, methodological difficulties in the purification of P_i in soil-plant matrices rendered $^{18}O_p$ that could not be isotopically analyzed.

A1.1.1 Measuring $^{18}O_P$

The isotopic composition of O in phosphate is defined as:

$$\delta^{18}O_P = \left(\frac{R(^{18}O/^{16}O)_{sample}}{R(^{18}O/^{16}O)_{standard}} - 1\right) 1000 \qquad \text{(Eq. A1.1)}$$

where R_{sample} and $R_{standard}$ refer to the $^{18}O/^{16}O$ ratio measured in the sample and standard of the Vienna standard mean ocean water (VSMOW), respectively, and values are reported as a delta (δ) in parts per thousand (‰). To measure $\delta^{18}O_P$, P_i must first be isolated and purified into silver phosphate (Ag_3PO_4). The silver phosphate analyte is then measured for O isotope ratios upon

conversion to CO using high-temperature reduction that is coupled to a continuous-flow isotope ratio mass spectrometer (Jiasi and Blake, 2014).

The most common technique to separate soil P into different fractions was developed by Hedley et al. (1982) where the initial extraction – resin-extractable P – targets the reactive P_i fraction that is rapidly available to plants. The soil is shaken with anion-exchange membranes to imitate root activity and P_i is continually removed from the solution onto the membranes which in turn causes more P_i to be released from the soil (Weiner et al., 2011). After uptake, P_i is transported throughout the plant, remaining in a form of P_i that is either free or bound in organic forms (such as phospholipids, ATP, DNA and RNA) (Hawkins and Polgase, 2000; Pfahler et al. 2013). Plant P_i is extracted by shaking plant tissue with weak trichloroacetic acid (TCA). TCA-soluble reactive P primarily contains P_i as well as some organic forms, such as sugar phosphates and phytate. However, these organic forms are not hydrolyzed during extraction, leaving the isotopic composition of P_i unaltered (Tamburini et al., 2014).

To analyze $^{18}O_p$, the precipitation of silver phosphate cannot be inhibited by interferences (e.g. Cl^- and Na^+ ions) or contain O species that alter $^{18}O_p$ values (e.g. nitrates, sulfates, other oxides and organic matter). P is a reactive compound that can form complexes with many substances via sorption, co-precipitation and redox cycling reactions (Jiasi and Blake, 2014). In polar deserts, plant tissues often accumulate high levels of calcium, magnesium and sodium (Maessen et al., 1983) from soils that are extremely low in P and although they are often devoid of organic matter, contain high amounts of chlorides and sulfates of magnesium and calcium that form complexes with P_i (Bliss, 1997). The most widely adapted approach to purify P_i removes contaminates using sequential precipitations of ammonium phosphomolybdate (APM) and magnesium ammonium phosphate (MAP) (Kolodny et al. 1983; Paytan et al., 2002; Liang and

Blake, 2007; Tamburini et al., 2010). This approach is suited to soils higher in organic material and oxides, and has also been used on TCA-soluble reactive P_i extracts from soybean leaves (Pfahler et al., 2013) and alpine grassland species (Tamburini et al., 2012). An alternate and less intensive approach that has been used on resin-extractable soil P_i and is suited for materials with less organic material, uses a single precipitation reaction of cerium phosphate (McLaughlin et al., 2004; Weiner et al., 2011). Many variations exist within each protocol to remove additional contaminates depending on the nature and chemistry of the sample. Commonly used treatments include organic adsorbents to remove humic and fulvic acids (e.g. DAX-8 Amberlite resin) (Tamburini et al., 2010), ion exchange resins to remove interfering anions and cations (e.g. AG 50W X8) (Tamburini et al., 2010; Weiner et al., 2011) and additional precipitation reactions to remove organic matter (e.g. magnesium-induced co-precipitation of phosphorus) (Karl and Tien, 1992; Blake et al., 2010). Additionally, the speed and volume of precipitations reactions are controlled to inhibit oxidation and precipitation of interfering compounds (Blake et al., 2010).

A1.2 Methods used to Extract and Purify $^{18}O_p$

Soil and *S. arctica* samples were collected from frost boils as described in Section 3.3.3.

A1.2.1 Extracting and Purifying Plant P_i

A1.2.1.1 Extracting TCA-Soluble Reactive Phosphate (TCA-P)

P_i was extracted from *S. arctica* tissue with trichloroacetic acid (TCA) following the procedure of Pfahler et al. (2013). Tissue samples were ground and 0.10 g of leaf, 0.22 g of stem or 0.36 g of root tissue were shaken at 4°C with 20 ml of 0.3 M TCA and filtered through 0.7 µm GF/F filters. Organic matter was removed by shaking the solution with 20 ml of DAX-8 Amberlite resin for 3 hours and filtering through a 0.2 µm polycarbonate filter. A subsample was collected to determine P concentration on a SEAL AutoAnalyzer AA1 (SEAL Analytical Inc.).

Table A1.1. Total P in plant and soil extracts and conditions required for $^{18}O_p$ purification protocols by Tamburini et al. (2010) and Weiner et al. (2011).

Sample	Total P	Tissue required for 30 µM P	P gains per 24 h	Time required for 110 µM P
	(mg L^{-1})	(g)	(µM)	(hr)
Leaf 0.10 g	4.37	0.02	-	-
Leaf 0.20 g	> 5.00	< 0.01	-	-
Stem 0.20 g	0.85	0.22	-	-
Stem 0.50 g	2.25	0.21	-	-
Root 0.20 g	0.48	0.39	-	-
Root 0.50 g	1.40	0.33	-	-
Soil 36 hours	0.90	-	19.4	136
Soil 48 hours	1.15	-	18.6	142
Soil 60 hours	1.60	-	20.7	127
Soil 5 days	4.36	-	28.2	94
Soil 7 days	3.62	-	16.7	158

A P concentration greater than 20 µM is required to purify TCA-P following the purification method in Section A2.1.2 by Tamburini et al. (2010). Various weights were sampled to determine the amount of *S. arctica* leaf, stem and root tissue needed to collect a TCA-P extract with a solution of 30 µM of P (20 µM + 10 µM excess) (Table A1.1). The amount of stem and root tissue required was 0.22 g and 0.36 g, respectively. Leaf extracts had high P concentrations and 0.10 g was chosen as an easily measurable amount of tissue that provided excess P.

A1.2.1.2 Purifying TCA-P using APM and MAP and Conversion to Silver Phosphate

A series of P precipitations was developed by Tamburini et al. (2010) and used to purify TCA-P on various plant species (Tamburini et al. 2012; Pfahler et al., 2013) for silver phosphate isotope analysis. This method was used to purify twenty-two extracts from Section A1.1.1. APM was precipitated overnight at low pH by adding 1 ml of concentrated sulfuric acid and 25 ml of

4.2 M ammonium nitrate buffer, placing in a 50°C shaking water bath and adding 40 ml of ammonium molybdate reagent (10 g of ammonium molybdate tetrahydrate and 90 ml of DDW). The APM crystals were separated on a 0.2 µm filter, washed with 0.6 M ammonium nitrate and dissolved with 30 ml of ammonium-citrate reagent (10 g citric acid, 140 ml of 14.8 M ammonium hydroxide, 300 ml DDW). MAP was then precipitated at high pH by adding 25 ml of acidified magnesia buffer (50 g magnesium chloride hexahydrate and 100 g ammonium chloride brought to 1 L with DDW and acidified to pH 1 with hydrochloric acid) followed by 7.5 M ammonium hydroxide to raise the pH to 8-9 and stirred overnight. The MAP crystals were separated through a 0.2 µm cellulose nitrate filter, washed with 1 M ammonium hydroxide solution and dissolved in 20 ml of 0.5M nitric acid. Cations were removed by shaking overnight with 6 ml of AG 50W X8 cation resin (Bio-Rad Laboratories Ltd.) then filtered through a 0.2 µm filter. Silver phosphate was precipitated slowly by adding 5 ml of silver-ammine reagent (P:Ag:NO_3:NH_4OH molar ratio of 1:100:300:750) and placed in an oven at 50°C for up to 48 hours.

A1.2.1.3 Purifying TCA-P using Rapid Micro-Precipitations of APM and MAP and Conversion to Silver Phosphate

A series of rapid micro-precipitations similar to the above method was developed by Blake et al. (2010) to purify phosphate from powdered rock. Twelve extracts from Section A2.1.1 were purified using a magnesium-induced co-precipitation of phosphate and micro-precipitations of APM, MAP and silver phosphate where volumes were constrained to less than 5 ml. Brucite was first precipitated by raising the pH to 10 with 0.5M NaOH, adding 0.45 g of magnesium chloride and sitting for two hours. The precipitate was separated by centrifugation and dissolved in 3 ml of 10 M nitric acid. APM was precipitated by adding 285 µL of 4.2 M

ammonium nitrate, placing in a 48°C shaking water bath and adding 1 ml of ammonium molybdate reagent (100 g molybdenum oxide, 400 ml of 15.6 M nitric acid, 14.8 M ammonium hydroxide and 1 L DDW). After thirty minutes, the remaining APM was precipitated rapidly by adding 6 ml of ammonium molybdate reagent and left overnight at room temperature. The APM crystals were separated with a 0.2 µm filter and washed with 0.6 M ammonium nitrate then dissolved with 1 ml of ammonium citrate reagent (Section A1.1.2) and 1 ml of deionized water. MAP was precipitated by adjusting the pH to 7, adding 0.25 ml of magnesia reagent (Section A1.1.2) and swirling samples while adding 7.5 M ammonium hydroxide to reach a pH of 8-9. After thirty minutes, the remaining MAP was precipitated rapidly by adding 1 ml of ammonium hydroxide and left overnight. MAP crystals were separated with a 0.2 µm cellulose nitrate filter and washed with 1 M ammonium hydroxide then dissolved with 1 M nitric acid. Cations were removed by shaking overnight with 6 ml of AG 50W X8 cation resin (Biorad Laboratories Ltd.) then filtered through a 0.2 µm filter. Silver phosphate was precipitated slowly by adding 0.75 ml of a silver-ammine reagent (P:Ag:NO_3:NH_4OH molar ratio of 1:10:30:75) and placing in an oven at 50°C overnight.

A1.2.1.4 Complications with Purifying and Converting TCA-P to Silver Phosphate

The methods used to purify TCA-P were unsuccessful in producing a pure, silver phosphate analyte (Table A1.2). The Tamburini et al. (2010) method was not effective in removing contaminants from the TCA-P extract. The micro-precipitates used in Blake et al. (2010) improved the removal of organic contaminates from 91 to 100% in APM crystals and increased the development of white MAP crystals from 30 to 67%. This was likely due to the removal of dissolved organic matter and interfering ions from the additional brucite co-precipitation and increase in control of each precipitation reaction by using small volumes

(Goldhammer et al. 2011). However, none of these treatments were able to remove contaminates that inhibit the production of yellow silver phosphate crystals. Black precipitates with traces of organic residues ranging in grade from grey to brown formed immediately after the silver-ammine addition.

A1.2.2 Extracting and Purifying Soil P_i

A1.2.2.1 Extracting Resin-Extractable Phosphate

Resin-extractable phosphate – the most plant available form of P – was extracted from soils following the protocol of Weiner et al. (2011). Briefly, 100 g of fresh soil, twenty-seven 7.5 cm x 3 cm anion-exchange membranes (607.5 cm^2, Western Ag Innovations, Saskatoon, SK) saturated in HCO_3^{2-} and 4 L of deionized water were shaken at 4°C for 5 days. Phosphate was cleaned and eluted from the membranes by subsequent overnight shaking with 400 ml DDW followed by 75 ml of 0.2 M nitric acid. A final shake with 20 ml of Supelite DAX-8 resin (Supelco, Sigma Aldrich) was used to remove organic material. A subsample was collected and analyzed by SRC Analytical (Saskatoon, SK, CA) to determine P concentration colometrically with the molybdate blue method using an AquaKem 200 Discrete Analyzer (Thermo Fisher Scientific).

The protocol was tailored to low phosphate arctic soils and various shaking periods were tested to extract a 110 µM P solution that had adequate phosphate for purification by Weiner et al. (2011) in Section A1.2.2. P gains per 24 hours ranged from 16.7 to 28.2 µM and an average shaking time of 125 hours was required to collect enough P for a 110 µM P solution (Table A1.1). For all subsequent extractions, 100 g of soil, twenty-seven 7.5 cm x 3 cm anion-exchange membranes and 4 L of deionized water were shaken for five days.

A1.2.2.2 Purifying Resin-Extractable Phosphate using Cerium Phosphate and Conversion to Silver Phosphate

Eight resin-extractable phosphate samples from Section A1.2.1 were purified by precipitating cerium phosphate using a method developed by Weiner et al. (2011). Cerium phosphate was precipitated overnight at 4°C by adding 3 ml of 4 M cerium (III) nitrate hexahydrate and 3 ml of 1 M potassium acetate buffer. The precipitate was rinsed three times with 0.5 M potassium acetate (pH 6) and dissolved in 2 ml of 1 M nitric acid. The cerium ions were removed by shaking overnight with 6 ml of AG 50W X8 cation resin (BioRad Laboratories Ltd.) and filtered through Whatman No. 4 paper. Silver phosphate was precipitated rapidly by adding 0.5 g of silver nitrate dissolved in 2 ml of DDW (Dettman et al. 2001).

A1.2.2.3 Purifying Resin-Extractable Phosphate using APM and MAP and Conversion to Silver Phosphate

Two resin-extractable phosphate samples from Section A1.2.1 were purified using a series of P precipitations described by Tamburini et al. (2010) in Section A1.1.2.

A1.2.2.4 Complications with Purifying and Converting Resin-Extractable Phosphate to Silver Phosphate

Similarly to TCA-P, the methods used to purify resin-extractable phosphate did not produce silver phosphate crystals (Table A1.2). Cerium phosphate formed in all samples using the methods of Weiner et al. (2011) but the success rate dropped to 67% during cleaning. Three of the precipitates became soluble after the addition of the potassium acetate washing solution even though it was acidified to pH 6 to prevent dissolution (McLaughlin et al. 2004). The speed of precipitation did not affect the success of silver phosphate and rapid precipitation rendered crystals contaminated with grey to white residues. The pH change from low to neutral pH to

Table A1.2. Success rate of individual precipitation reactions used to purify soil and plant P_i extracts.

Method Source	Step	Success Rate	Failed Samples	Total Samples
		%	*n*	*n*
Plant				
Tamburini et al. (2010)	APM	91	2	22
	MAP	30	14	20
	Ag_3PO_4	0	6	6
Blake et al. (2010)	Brucite	100	0	12
	APM	100	0	12
	MAP	67	4	12
	Ag_3PO_4	0	8	8
Soil				
Weiner et al. (2011)	$CePO_4$	63	3	8
	Ag_3PO_4	0	5	5
Tamburini et al. (2010)	APM	0	2	2

form cerium phosphate can encourage oxide precipitation and adsorption of organic compounds onto the newly-formed oxides (Tamburini et al. 2014). The APM precipitate in Tamburini et al. (2010) was developed to avoid a large pH adjustment but despite this precaution APM did not crystallize and was inhibited by the formation a large, white residue that could not be manipulated in subsequent steps.

A1.3 Further Approaches

Using ion chromatography systems to separate P_i could provide an alternative to using sequential precipitation reactions for problematic materials. Anion and cation resin columns have been used to remove interfering ions from powdered rock and water samples (Blake et al., 2010) but have not yet been integrated into plant and soil methods. After P_i extraction, sample volume could be reduced (through evaporation or magnesium induced co-precipitation of phosphorus)

(Blake et al. 2010) and injected into a simple anion chromatograph (Goldhammer et al. 2011) or automated ion chromatography system (e.g. Dionex ICS-2100) to separate out P_i. For samples heavy in dissolved organic material (i.e. plant tissue), a pre-treatment may be necessary to remove material before separation. Afterwards, samples could be converted directly into silver phosphate – eliminating the need for all prior phosphate precipitation reactions and additional resin treatments.

Appendix 1 References

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APPENDIX 2

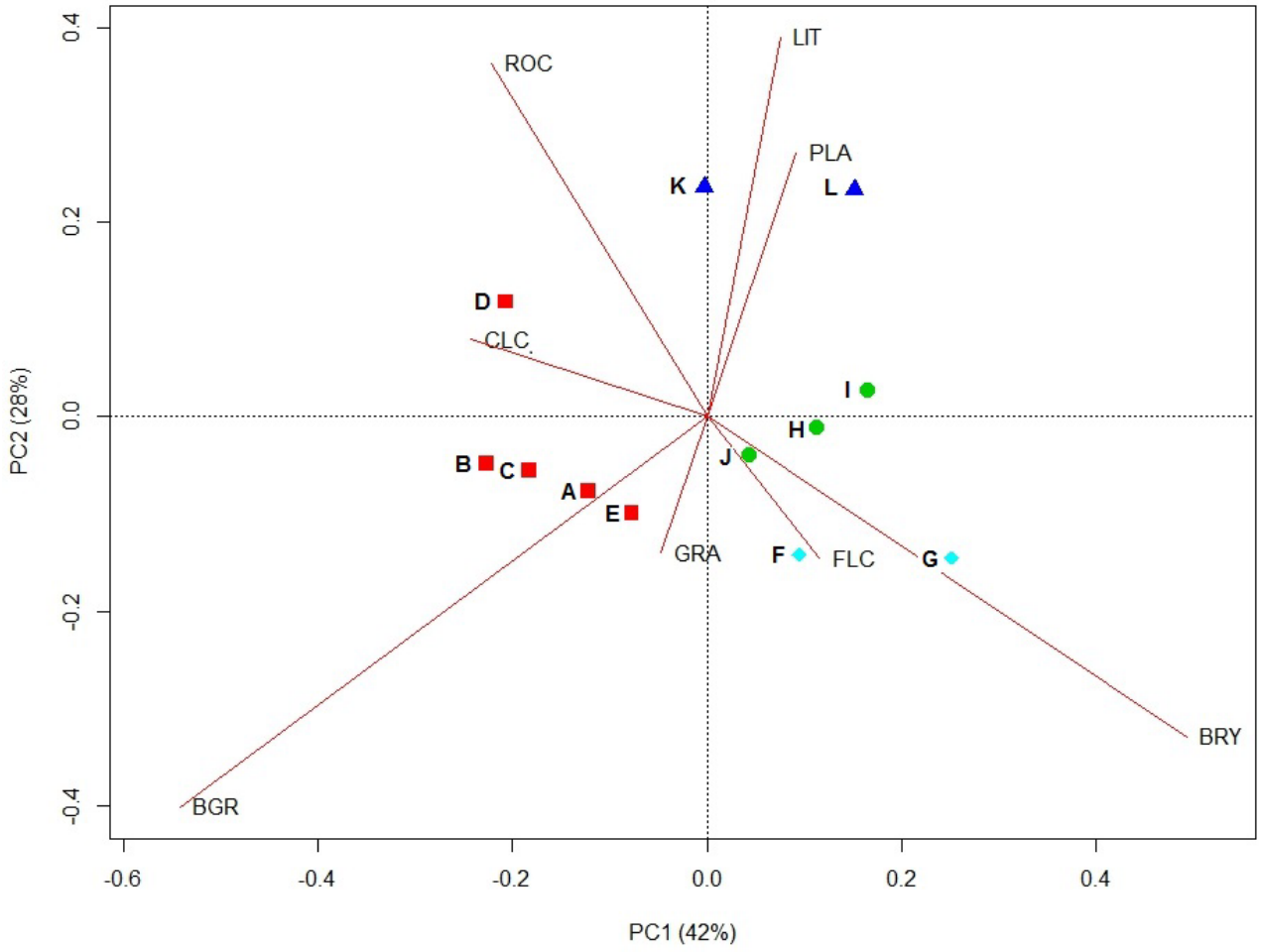

Figure A2.1. Ordination of average surface cover characteristics within blocks on granite parent material at Dome site used to define surface cover groups I-IV in Chapter 3. Surface characteristics are defined as rock (ROC), gravel (GRA), bareground (BGR), crustose lichen (CLC), fructose lichen (FLC) bryophytes (BRY), litter (LIT) and plant (PLA) in % cover. Principal component analysis was performed in R (ver. 3.2.2, R Core Team, 2014) using the rda function within the Vegan package. The ordination was overlain with group clusters that were defined with Ward's minimum variance using the hclust function.

APPENDIX 3

Table A3. Total plant, *S. arctica* and litter cover on control and diapir frost boils of surface cover groups on granitic parent material. Values are means and (standard errors) of each frost boil type within surface cover groups.

Surface Cover Group	Total Plant	*S. arctica*	Litter
		— % cover† —	
Control			
I	6.1 (0.5)	5.6 (0.5)	9.0 (1.1)
II	5.4 (0.6)	5.0 (0.8)	11.8 (2.0)
III	8.1 (2.4)	7.5 (2.7)	13.4 (2.6)
IV	10.2 (0)	10.0 (0.2)	16.3 (1.7)
Diapir			
I	7.1 (1.2)	6.2 (1.2)	15.1 (3.3)
II	4.9 (0.5)	4.2 (1.2)	8.2 (0.2)
III	11.7 (2.8)	10.0 (3.0)	18.2 (3.9)
IV	15.5 (2.0)	14.4 (2.7)	31.6 (3.9)

† Classes of surface cover are % cover averages of 25 cm by 25 cm quadrats (n=9) on each frost boil.